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CONTENTS

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THE INDIAN JOURNAL OF GENETICS AND PLANT BREEDING

Vol. 20, No. 2

TABLE OF CONTENTS

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VOL. 20 | MAY 1960 | No. 2

SOME LIMITATIONS OF RADIATION GENETICS AND PLANT BREEDING

W. M. MYERS*

THERE has been in recent years a great amount of attention directed to the potentialities of ionizing radiations and other mutagens for induction of useful mutations in plant improvement. With the optimistic writing of Gustafsson and his associates in Sweden, with the vastly increased availability of facilities and materials for application of mutagenic agents, and with relatively large amounts of money dedicated to studies of peaceful uses of atomic energy, plant breeders all over the world have used irradiation with the hope of producing useful mutations. Enthusiasm has been particularly high in areas where conventional plant breeding is less well developed and where needs for increased food production are great. Mutation breeding has been grasped as a sort of magic wand by which the plant breeder can hope to span, in a few years, the developments in other areas by conventional plant breeding during half a century. It is looked upon by some as a panacea rather than as a possible plant breeding tool, still to be evaluated and developed. In justice to specialists in radiation genetics and breeding, it is not usually they who have been overly enthusiastic about it. Rather the fault lies more with plant breeders who are not thoroughly familiar with its potentialities and limitations and with ill-informed directors of research and politicians speaking for public consumption.

When, in the midst of glowing optimism, one sounds a note of conservatism, he may be labelled as old fashioned and accused of "throwing cold water". Yet it has been my observation that there is today more need to point out some limitations of radiation breeding than to emphasize its potentialities. The need is not to encourage mass usage of radiation in plant breeding. Rather the problem is to insure its proper usage so that its potentialities can be reliably evaluated, technique for successful use can be developed, and waste of critically short plant breeding personnel and facilities can be avoided.

Mutation breeding has two objectives, i.e. (1) to increase genetic variability and (2) to produce a specific mutation that will confer a desired character on an otherwise superior variety.

Genetic variability is, of course, the life blood of plant breeding. No amount of selection can bring about significant genetic advance in a population in which too little genetic variability occurs. Genetic variability has its origin from mutation and it has been known since the work of Muller and Stadler that mutation frequency can be increased substantially by various mutagenic agents, including ionizing radiations. It is logical, therefore, to think of irradiation as a means of increasing genetic variability. But it is appropriate to examine the relative potentiality of induced variability (mutation) versus already existing variability.

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In spite of the great amount of research already done on natural and radiation induced mutations, we still know very little about the frequency or kinds of mutations even in such intensively studied organisms as Drosophila and maize. Wallace and Dobzhansky (1959) have drawn some generalization from available evidence. In maize the natural mutation rate per gene per generation ranges from 1 per 10,000 individuals to 1 per million. In Drosophila, using data from chromosomes II and III, the average spontaneous rate per gene per generation is estimated at about 1 per 100,000 while the rate with 1000 r units of irradiation is about 1 per 10,000. Wallace's and Dobzhansky's estimates might be carried a step further by assuming that an organism has 10,000 gene loci that are capable of mutating. In that case there should be an average of one mutation per individual per generation, exposed to 1000 r of radiation. This, at first glance, might seem to be a fairly high rate of increase in genetic variability. However, we must bear in mind two limitations. First, a mutation of one gene in 10,000, unless it controls some major and essential process, would not probably have a measurable effect upon the variability in a population. Second, according to Wallace and Dobzhansky, about one-fifth of all observable mutations are lethal and four-fifths range in effect from sub-lethal to nearly neutral. We do not really know how frequently a mutation may be superior to its normal allele. However, considering that existing "normal" alleles have survived through a great many generations of natural selection, it has been assumed that new mutations will very rarely be better than "normal" under the existing conditions of external environment and genetic background. Actually mutation experiments, so far as they have gone, bear out this assumption. A vast majority of mutations, natural or induced, are at least partially deleterious. "Desirable" mutations are very rare indeed. If one, therefore, considers both the infrequency of mutation and the very low proportion of "desirable" mutations among those obtained, it seems evident that in a single cycle of irradiation the amount of increase in desirable genetic variability will be very small.

Contrast this with the enormous amount of natural genetic variability that has accumulated in most of the species of crop plants during 50 centuries and 5,000 generations or more since they originated and were domesticated. From the writings of Vavilov and others and from the limited samplings that have been made in "centres of genetic diversity", it seems evident that even on a world wide basis plant breeders have only a limited sample of the total natural genetic variability that exists. Within any single plant breeding program, the individual breeder has only a small part of that limited sample. Finally, few plant breeders have made full use, by conventional and according to some, old fashioned, plant breeding procedures, of the genetic variation available in their respective populations. Obviously, in no crop plant have plant breeders yet "scratched the surface" in utilizing the genetic variability existing within the species in nature.

Added to the genes in the cultivated species are the vast array of genes in related species. Classical examples of transfer from a related wild species of genes not known to be available in the cultivated species are found in wheat, potatoes, tobacco and other crops. Modern advances in embryo culture, polyploidy, use of bridging species, alien chromosome and gene substitution and other techniques are expanding the range of species from which desired genes can be borrowed.

With such a relative wealth of natural genetic variability, in contrast to the expected infrequency of favourable induced mutations, the plant breeder cannot afford at this stage in the science of radiation genetics to devote part of his resources to use of radiation for increasing genetic variability. An exception to this generalization is the case in which some character is required for which no known gene exists in the species or in a sufficiently close relative. The student of genetics who has read the writings of Von Sengbusch during the second and third decades of this century is impressed

with the intensive search for and eventual discovery of mutations required to convert annual *Lupinus* spp., to cultivated crops. The frequency of such mutations might well have been increased by use of mutagenic agents. But, with the present degree of utilization of existing natural variability, such cases are the exception rather than the rule.

The second objective of mutation breeding, namely to correct a single weakness of an otherwise desirable variety, seems less likely of accomplishment than was supposed at the time of some of Konzak's earlier writings. Experience has indicated that "clean" mutations are not often obtained. Usually the so-called mutations have been associated with, or perhaps in some cases the result of, multiple chromosomal derangements. Furthermore, favourable response attributed to a single gene is normally the result of action of that gene plus a properly balanced set of modifiers. A new "raw" mutation may generally be expected to upset the fine genetic balance established by selection. Therefore the new mutation, even when "favourable", may actually be favourable only after a new genetic balance has been established. Admittedly this borders on theoretical expectation rather than realization from experiments. But we might cite the example of "Sanilac" field peas, a new variety recently released in Michigan. It is a bush type variety derived from "Michelite" by irradiation to induce the bush type habit followed by back-crossing to Michelite and further selection (Down and Anderson, 1956).

In sounding these notes of conservatism, we should not overlook the role of polyploidy in conditioning response of a species to mutation breeding. Available evidence indicates that chromosomal derangements are a common, perhaps the most common, result of irradiation. In diploid species or in very old polyploids in which extreme genic differentiation between genomes has occurred, most of these derangements will not persist if the organism reproduces sexually. Many, particularly the deficiencies and duplications, are lethal when homozygons. On the other hand allopolyploids in which differentiation of the genomes has not progressed too far can tolerate derangements including deficiencies and duplications. When, in such a species, the desired character depends upon the absence of a particular gene irradiation may be a tool for improving a variety for that particular character. Induction of awned types in awnless varieties of bread wheat by Swaminathan and his associates is an example of this kind. We should emphasize, however, that the precise use of radiation as a tool in such a case requires more knowledge of the cytogenetic make up of the species and genetic basis of character determination than is available for almost any other species or character.

Lest these remarks be interpreted as arguments against further studies of radiation breeding, let us consider some information that is needed in the more precise use of radiation as a plant breeding tool.

We have already referred to the lack of information on frequency of different kinds of mutations. Until much more data of this kind are available, the plant breeder cannot use mutation breeding as a precise tool. This is perhaps the place to point out a major weakness of a majority of the mutation breeding experiments reported to-date. They have been concerned with self-pollinated crops and, assuming that selfing is the rule, no steps have been taken to preclude the possibility of natural crossing. Yet in almost every self-pollinated species there is some natural cross-pollination, varying from less than 0·5 to as much as 3 or 4 per cent. Male sterility, induced in the first generation following irradiation, markedly increases the incidence of cross-pollination. With the low expected frequency of mutation, it is evident that contaminations due to cross-pollination could vitiate the results of any experiment in which self-pollination is not rigidly enforced. My colleague, R. S. Caldecott, is in fact of the opinion that most of the so called mutations for disease resistance and other desired characters reported in wheat, oats and barley were actually the result

of natural crossing. He has extensive data from oats which seem to support his contention in this regard.

Because of the rarity of mutation for a particular locus, much more information is required on how to increase the frequency of favourable changes. Ultimately we might hope to be able to direct mutation or at least to accomplish changes at some loci while not affecting others. This will require much more extensive investigations of the mutagenic tools and also a more critical evaluation of kinds of mutational changes than is possible with available knowledge and techniques. Of particular interest in this connection is the question of whether or not kinds of mutations are possible which do not occur naturally and whether certain loci which do not mutate naturally can be induced to do so.

Plant breeders will almost certainly reach a point some time in the future when they must resort to induced genetic variability. This may occur all too soon through loss, due to man's own activities and negligence, of the vast storehouse of variation that has accumulated through the centuries. If not, it may still occur in time due to use of all existing variability by plant breeders, although in my opinion that time is still several generations of plant breeders in the future.

In any event, radiation breeding is a potential tool that deserves extensive study. The problem is not whether but where it should be studied. In this connection, I believe that study should be limited to a few institutions where personnel and facilities are available to do the kind of research required, including growing and evaluating very large populations of plants under rigidly controlled conditions. As a general rule, the plant breeder cannot afford today to adopt it as a breeding method. To do so will result in wasting the time and facilities so used. The plant breeder who studies mutation breeding must do so primarily to evaluate and develop the tool. Unless he has more than sufficient resources for plant breeding by conventional methods, he cannot afford to divert part of his resources to mutation breeding.

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INTROGRESSION BETWEEN *HYPARRHENIA CYMBARIA* STAPF AND *H. PAPILLIPIES* ANDERSS. IN DISTURBED HABITATS OF ETHIOPIA

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As more and more cases of introgressive hybridization have been analyzed (Anderson, 1949; Heiser, 1949; Epling, 1947), it has become increasingly clear that two species may hybridize rather extensively when man or any other agent disturbs the habitat, even when their hybrids and back crosses are not commonly found in nature. Much of the evidence from sympatric introgression has shown that such species are kept apart by ecological and internal barriers of various kinds (Anderson, 1949; Epling, 1947; Stebbins, 1950). In old, well-established floras the whole system is closed since most of the plants have evolved together. It is not until ecological barriers are broken down that there will be appreciable hybridization between sympatric species. When man disturbs the habitat by burning, pasturing, cutting and tillage, he produces varying ecological niches in which some of the segregation products of introgression may be at a selective advantage. Anderson (1948) referred to this phenomenon as " Hybridization of the habitat "; its evolutionary importance has been discussed by Anderson (1949), Grant (1953), Stebbins (1950), and Anderson and Stebbins (1954).

Many genera of plants have now been investigated and the importance of introgressive hybridization has been demonstrated (Anderson, 1949; 1952; 1954; Grant, 1953; Desmarais, 1952; Hall, 1952; Heiser, 1949) in various field studies. However, the bulk of this work has been conducted in North America and Europe; relatively little is known about the evolutionary mechanisms of plant species from disturbed habitats in Asia and Africa. In countries such as Ethiopia, India, Egypt, Iraq and China, where agriculture is quite old, natural habitats have been repeatedly disturbed and one may expect high frequencies of introgressive hybridization between certain species of plants.

In a recent visit to Ethiopia, the junior author was impressed by the extensive plant-to-plant variation of the genus *Hyparrhenia* of the grass tribe Andropogoneae. The variation pattern of these populations seemed to be discordant (Anderson, 1951), a general characteristic of strongly introgressed populations. Herbarium specimens were prepared from the *Hyparrhenia* populations; field notes were recorded separately for three highly disturbed habitats. The bulk of these specimens could be identified to the nearest species, but a sizable number appeared to be intergrading toward *H. cymbaria* Stapf and *H. papillipies* Anderss. The present paper deals with the morphological analysis of these plant collections.

Field notes

Herbarium specimens (" mass collections ") of different *Hyparrhenia* species were prepared from three very different sites in Ethiopia. The field notes pertaining to these collections are outlined below:

(a) *Dembidolo airstrip collection:*

The material was collected by Edgar Anderson and Hugh Rouk in November, 1957, from the edge of the airstrip at Dembidolo in Western Ethiopia. The airfield, located on the edge of an old village, had apparently been levelled and had been repeatedly mowed. The habitat had consequently been greatly and repeatedly

disturbed. Twenty-one plants were collected at random along a path near native huts in the following manner; one plant was picked nearest to every right footstep. A few very extreme plants (extremes approaching each parental species or recombinants from two or more species) were also added to this collection.

The Hyparrhenias of this site were extremely variable, though to the junior author it did not appear like a simple case of introgression. The grasses were in full flower. Brilliant wine purple, lemon yellow and clear terracotta colours were common in addition to the tawny and amber shades characteristic of most Andropogoneae when in bloom. Some of the more brilliant colour types obviously occurred again and again along the pathway.

Seven species of *Hyparrhenia* were identified from this collection. These species were *H. ruffa* Stapf, *H. pusilla* Stapf, *H. hitra* Stapf, *H. umbirosa* Anderss., *H. filipendula* Stapf, *H. confinis* Anderss. var. *pallita* and *H. confinis* Anderss.

(b) *Old Limmu road collection:*

Thirty-two plants were collected by Edgar Anderson and Asnake Getachu in November, 1957, along the shifting trackways of the old highway leading to Limmu, one mile northeast of Jimma, Kaffa Province, Ethiopia. The roadway was at least a century old and had once been well defined with *Euphorbia* hedges. The footpaths and the tracks made by bundles of firewood being pulled to market had shifted back and forth across this area, and the entire area had been repeatedly, and closely, grazed. Also, the bundles of firewood dragged to market had harrowed the surface, and the trackways had been repeatedly relocated. A sample of every flowering clone of Andropogoneae was collected from about a mile along the roadway. All proved to belong to the genus *Hyparrhenia*.

Some days before this collection was made, detailed studies of population samples from other sites had convinced the junior author that *H. cymbaria*, in spite of its strikingly different size and habit of growth, was perhaps responsible for much of the variation in these weedy *Hyparrhenias*. Therefore, in collecting the Limmu samples *H. cymbaria* was collected from the hedgerows along the roadway in addition to the collection of every flowering clone of Andropogoneae from the actual roadway. The tall, gracefully drooping *H. cymbaria* grew here and there in semi-shaded spots in the milk-bush (*Euphorbia* species) hedgerow. In this old well established hedgerow there was little variation from plant to plant, even when carefully examined with a hand lens.

The following species of plants were identified: *H. diplandra* Stapf, *H. hirta* Stapf, *H. filipendula* Stapf, *H. rufa* Stapf, *H. cymbaria* Stapf, *H. papillipies* Anderss., and also plants intergrading between the last two species.

(c) *Budabuna forest collection:*

Eight plants were collected by Edgar Anderson in October, 1957, along a shady roadside (two miles east of Jimma, Ethiopia) leading through a coffee plantation forest and adjacent sunny pasture. The selection of these eight plants was very subjective since only such plants were collected which appeared to have characteristics of *H. cymbaria* Stapf and *H. papillipies* Anderss., (or closely similar species).

The habitat along the roadside was greatly disturbed and the vegetation constantly changed because of grazing, passage along the road, and the periodic cutting out of vegetation between the coffee bushes in the forest.

These eight specimens were identified by Dr. George B. Van Schaack as intergrading forms between *H. cymbaria* and *H. papillipies*.

Material and Methods

A comparative morphological study was conducted of thirty plants which seemed to show varying degrees of intergradation towards *Hyparrhenia cymbaria* and *H. papillipies*.

These plants were collected from disturbed habitats in Ethiopia, outlined above. Nearly all of the herbarium specimens examined bore several racemes. This was taken advantage of in two ways. Firstly the examination of the variation patterns both within and between plants made it possible to choose characters which were relatively independent of the environmental effects, and secondly in selecting racemes for measurements great care was taken to choose those which were typical of the plant specimens. Comparable parts of different plants were studied; most of the obviously variable morphological characters were either measured or given a numerical value (Table 1). Morphological data were recorded from five leaves and five inflorescences from each herbarium specimen, and the average values were calculated for each character.

Table 1

Class intervals for the morphological analysis of introgression between H. cymbaria *and* H. papillipies

Morphological characters		Index values		
		0	1	2
1 Spatheole	— length mm.	0–30	31–40	41–50
2 Raceme	— length mm.	3–13	14–20	21–30
3 Ligule	— pubescence	High	Medium	Absent
4 Glume	— pubescence	Slight	Medium	High
5 Peduncle	— pubescence	Slight	Medium	High
6 Raceme	— width	Thin	Medium	Thick
7 Peduncle	— length mm.	0–20	21–30	31–60

Observations

A preliminary morphological study revealed that the most conspicuous and prevalent plant to plant variation was with respect to the following morphological characters (Fig. 1, 2).

(1) *Pubescence near the ligule:*

The presence or absence of tubercle-based hairs near the ligule of the leaf was scored in three grades: absent, slightly pubescent and very pubescent. *H. cymbaria* has many hairs, while *H. papillipies* has either few or no hairs.

(2) *Length of the spatheoles*:*

The length of the spatheole was measured to the nearest millimeter. *H. cymbaria* has spatheoles that range in length between 8-16 mm. while in *H. papillipies* the length of the spatheoles was observed to be 20-54 millimeters.

* Spatheole: a small bract-like envelope beneath the raceme in certain grasses.

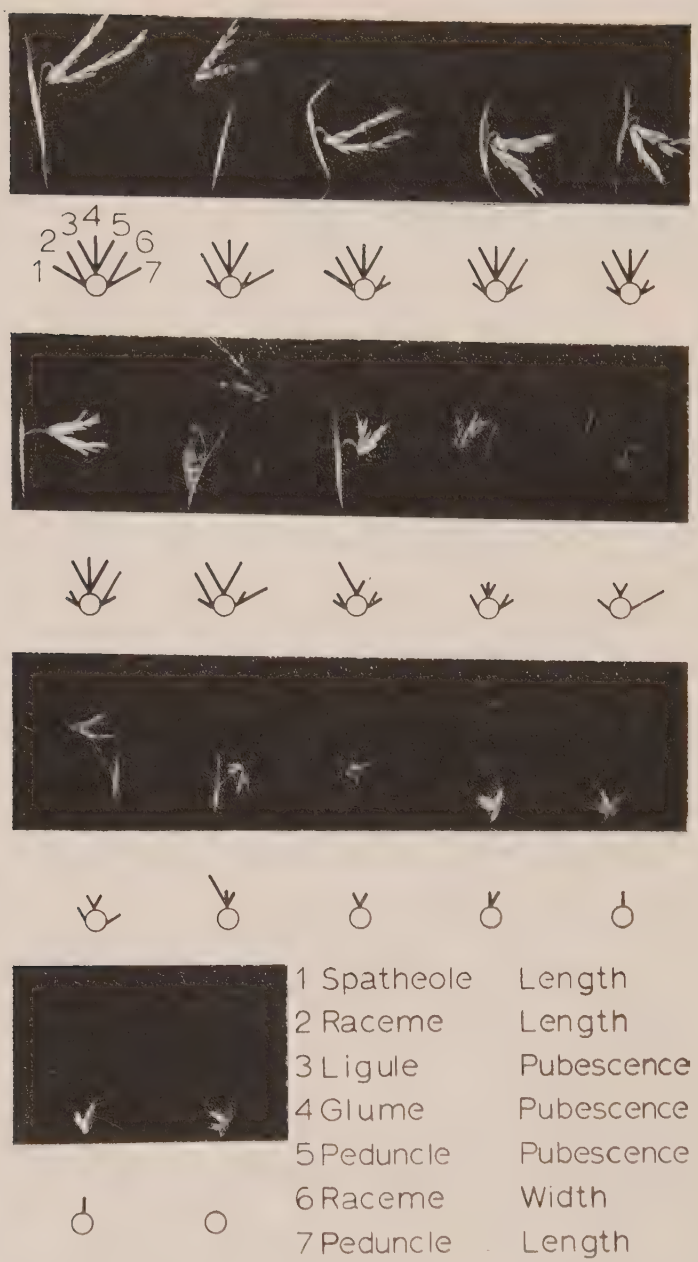

Fig. 1. Photographs of portions of *Hyparrhenia* plants used in analyzing introgression between *H. cymbaria* and *H. papillipies*. Comparable portions of seventeen plants are shown. First, third, fifth, and seventh rows show photographs of plants and second, fourth, sixth, and eighth rows are their corresponding metroglyphs. First row on extreme left *H. papillipies*, seventh row on right *H. cymbaria* and other plants intermediate between these two species.

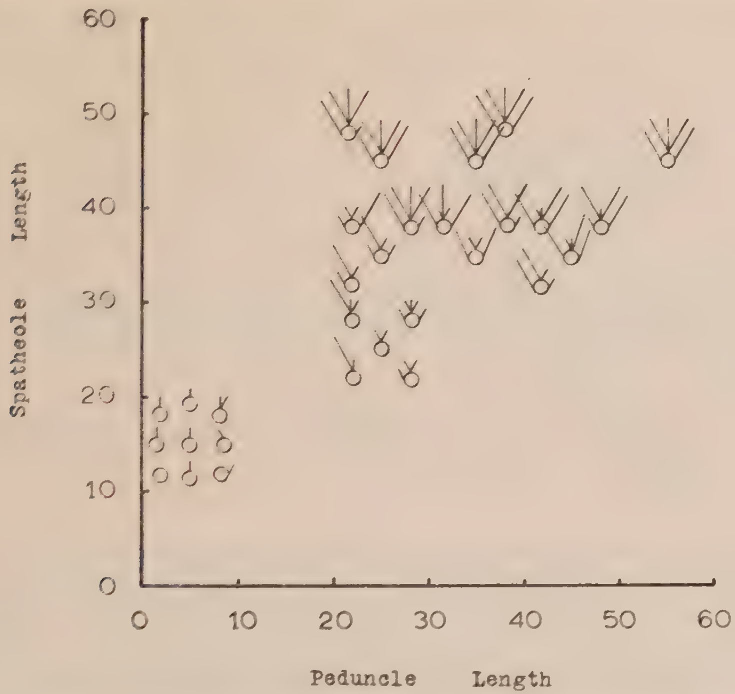

Fig. 2. Introgression between *H. cymbaria* (lower left) and *H. papillipies* (upper right) as shown by 30 plants collected along the old Limmu Road (shown in detail in Fig. 1) and at the Budabuna forest nearby, Jimma, Kaffa Province, Ethiopia. Each of the 30 glyphs represents a single plant, each scored for seven characters as denoted in Table I. Only introgression could explain the loose overall association between spatheole, raceme, and peduncle lengths, raceme width, and pubescence patterns of the ligule, glume, and peduncle shown by this diagram.

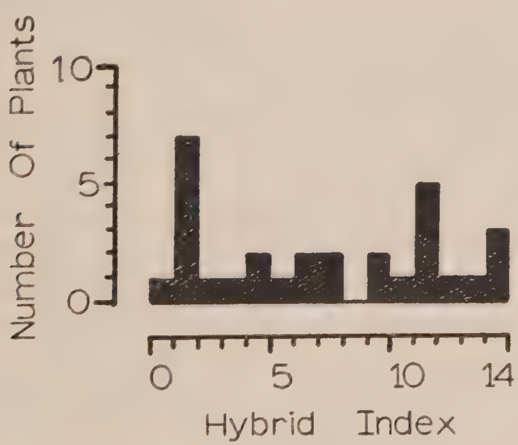

Fig. 3. Frequency distribution of hybrid index derived from the data in Fig. 2, as described in the text. A value of 'O' indicates extreme *H. cymbaria*, a value of '14' an extreme *H. papillipies* for the seven measured and scored characters of Fig. 2. Analysis of Figs. 2 and 3 indicates strong introgression of *H. cymbaria* into *H. papillipies* and a variability in *H. cymbaria* which might be due to slight introgression from *H. papillipies*, or some very similar species.

(3) *Length of the peduncle:*

The length of the peduncle was measured to the nearest millimeter. The length of the peduncle was observed to show a range of 4-6 mm. and 20-60 mm., in *H. cymbaria* and *H. papillipies* respectively.

(4) *Pubescence of the peduncle:*

This character was scored in three grades, i.e., few, intermediate and many hairs. *H. cymbaria* has few hairs, while *H. papillipies* has many hairs.

(5) *Length of the raceme:*

The length of the raceme was measured to the nearest millimeter. The range in length of the raceme was observed to be 5-10 mm. and 15-30 mm., in *H. cymbaria* and *H. papillipies* respectively.

(6) *Pubescence on the glumes:*

This character was scored in three grades: few (*H. cymbaria*), intermediate and many hairs (*H. papillipies*).

(7) *Width of the raceme:*

The width of the raceme was measured in three grades, i.e., thin, medium and thick. *H. cymbaria* was observed to have thinner racemes in comparison to those of *H. papillipies*.

In order to obtain an over-all understanding of how these morphological characters were varying, and how the variations of each character were more or less associated with those of the others, the data were plotted (Fig. 1, 2) as metroglyphs (Anderson, 1957). Of the seven morphological characters studied, length of spatheole and length of peduncle were used as the ordinate and the abcissa of the scatter diagram, because they were clearly two characters which were not only varying widely amongst these plant specimens, but also could be measured in a series of grades. The other five characters are indicated by rays emerging from each metroglyph. Each glyph represents one plant, and its position indicates its value for the characters used on the axis. The absence of a ray, a small ray and a long ray indicates the absence, intermediate, and a high value of a given character. Furthermore, in drawing the diagrams for each of these seven characters, those extremes which tend to be associated together were diagrammed with long rays while the opposite values were designated with no rays on the glyphs (Table 1).

By assigning index values (0, 1 and 2 to absent, short, or long rays respectively) to different rays representing varying grades of the morphological characters plotted on the scatter diagram (Fig. 2), and also by allotting values (0, 1 and 2) for the characters used on the axis, a hybrid index (Fig. 3) for the population was prepared (see Anderson, 1957, for details as to hybrid indices). By summing the scores for each of the seven characters, the plants were given an assessment as follows: *H. cymbaria* (0-2), intermediate (3-9) and *H. papillipies* (10-14). Figure 4 shows the frequency histogram for the collections on this sevenfold hybrid index. Rayless glyphs represents one extreme (score values 0), while the other extreme is represented by the index of 14.

Character association analysis for the seven morphological characters indicated that these characters are associated in two well defined complexes.

(a) *H. cymbaria* complex:

This complex is characterized by the association of the following characters; short spatheoles, short and thin racemes, peduncles shorter than the spatheoles, slight

or no pubescence on glumes and the upper halves of peduncles, and tubercle-based hair near ligule.

(b) *H. papillipies* complex:

This complex, on the contrary, is characterized by the association of the following characters; thick and long racemes, that are exserted out of long spatheoles, pubescent peduncles and glumes, few (or absent) tubercle-based hairs near the ligule, and many fertile spikelets on the racemes.

Many plants showed various combinations of these seven morphological characters. Furthermore, the variation in these intergrading plants was *in the direction* of both *H. cymbaria* and *H. papillipies*. The loose association between all seven morphological characters and the recombination between certain characters could only be explained by assuming hybridization between *H. cymbaria* and *H. papillipies* or between two species with similar combinations of characters. Since no such species are known, the most likely hypothesis is that hybridization and subsequent introgression between *H. cymbaria* and *H. papillipies* are involved.

Discussion and Conclusions

The number of plant specimens used in the present study, in preparing the pictorialized scatter diagrams for the hybrid swarm of *H. cymbaria* and *H. papillipies*, were small. Anderson (1943) and Woodson (1947) have shown that relatively few specimens are needed to indicate the trend of variation of a given plant population. Recently, Duman and Kryszczuk (1958) have suggested the presence of introgression in the *Carex-Stans-Bigelowii* Complex on the basis of the morphological study conducted on thirty herbarium specimens. As indicated by Anderson (1954), if we are considering seven characters, each on twenty-five specimens, then the total number of basic observations is one hundred and seventy-five. If, furthermore, we use a method such as metroglyphs, which is capable of demonstrating the interrelationships between all seven characters, then the analysis may become highly significant even for very small samples. In customary statistical work we have a few observations on each of a large number of individuals, while in metroglyph analysis we have many observations on each of a few individuals. In both instances, the total number of observations is large enough to yield reliable results.

Introgression between *H. cymbaria* Stapf and *H. papillipies* Anderss., would be another case of natural hybridization in disturbed habitats. It is likely that the variable populations of *Hyparrhenia* came originally from hybridization between *H. cymbaria* and *H. papillipies*. Furthermore, the primary hybrids backcrossed to each of the two parental species, thus providing varying recombinations of the characters of these two species.

Whether the amount of hybridization and backcrossing estimated for the two populations of *H. cymbaria* and *H. papillipies* is typical for the entire area of the disturbed habitats of Ethiopia, cannot be ascertained until more population studies have been made.

Celarier and Harlan (1957) have reported the presence of apomixis in certain members of the tribe Andropogoneae. Emery and Brown (1958) have reported the presence of apomixis in *H. rufa* and *H. hirta*, and it seems likely that it is also a common phenomenon in other species of *Hyparrhenia*. We could not observe much variation in the herbarium specimens of the other species, collected in the disturbed habitats of Ethiopia. It may be due to first, our few samples of these two species; secondly to the fact that the material collected from the same locality may have come

from the same clone, and thirdly at least some of the species may be reproductively isolated from each other on account of apomixis.

In retrospect, the most striking feature of this study is the dramatic contrast between the collections from the Dembidolo airstrip and from the Limmu roadway. The former was ecologically a seemingly uniform habitat. The variability and heterogeneous nature of the population are apparently due to ancient hybridization followed, at least in part, by apomixis. Along the Limmu roadway, every Andropogonaceous grass of flowering size had been collected from the most disturbed area of the roadway from a length of about a mile. Every plant proved to be a *Hyparrhenia*. As a whole these weeds looked to casual examination more like each other than did those in the tightly packed flowering sward adjacent to the airstrip. Yet, each clone (when examined) was different (Fig. 1 and 2); the entire collection strongly indicates that the bulk of the variability is introgressive. It comes from the uniform, graceful plants of *H. cymbaria*, which seemingly have little or nothing to do with the low weeds of the roadway. Though the collections are small, they are selectively collected and have been rigorously examined. They are a warning of the biological complexities we must expect to find in African genera like *Hyparrhenia*. Polyploidy, apomixis, and introgression in areas where there have been waves of human interference stretching through millenia can produce mingled order and complexity on a vast scale, yet intricate in detail.

Summary

1. Three collections of *Hyparrhenia* from disturbed habitats of Ethiopia were studied and nine species were identified, as follows: *H. pusilla*, *H. hirta*, *H. umbirosa*, *H. filipendula*, *H. confinis* var. *pallita*, *H. confinis*, *H. diplandra*, *H. cymbaria* and *H. papillipies* and intergrading plants between the last two species.

2. Association of seven morphological characters was analyzed and by the use of the metroglyph analysis the presence of a hybrid swarm with introgression between *H. cymbaria* and *H. papillipies* was postulated.

3. The significance of these findings is discussed. It is suggested that apomixis, polyploidy, and introgression may be expected to produce complicated variation patterns. In regions, such as Ethiopia, where waves of human interference have operated over millenia, we may well expect to find a mingled order and complexity, intricate in the details of its patterns.

Acknowledgement

The authors wish to thank Drs. Jack R. Harlan, George B. Van Schaack and the late Robert P. Celarier for identification of some specimens and to Hugh M. Rouk and the staff and students of the Jimma Agricultural and Technical High School, Jimma, Ethiopia, for equipment, assistance, and stimulating companionship.

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A STUDY OF SURVIVAL IN A MIXTURE OF FOURTEEN VARIETIES OF WHEAT

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Competition among genotypes has long been of interest to the student of evolution of plant and animal species and the important role played by such competition in natural selection has clearly been pointed out by Haldane (1932). The breeder of autogamous plants, too, is interested in the effect of such competition on the change in proportion, over a number of years, of different genotypes grown in mixed cultures. For, such a situation is met with when the progeny from a cross is handled as a bulk for a number of segregating generations with little or no attempt at selection. It has been urged against this method of breeding that it may lead to the loss of potentially valuable genotypes capable of giving high yields if grown in pure stands. This problem has been studied quite extensively in the past, usually in artificial mixtures of several varieties of various cereal crops, especially wheat and barley (Harlan and Martini, 1938; Laude and Swanson, 1942; Suneson and Wiebe, 1942 and Suneson, 1949). These studies have, in general, been restricted to competition among varieties belonging to a single species, though recently, Sakai (1955) has compared diploids and polyploids and has shown that alloploids at least are likely to be more competitive than the parental species. In India, a number of wild and cultivated species of wheat occur. Factors determining the adaptability of species to the soil, climate and other environmental factors as well as human preferences have undoubtedly played a part in the greater prevalence of particular varieties of the cultivated species. It was felt that a brief description of the results of an experiment, involving competition between varieties belonging to all the three chromosome groups of wheat, carried out at this division over the years 1943-1949, would prove of some interest to those working in wheat.

Material and Methods

Fourteen varieties, belonging to eleven species of wheat, were taken up for the present study. A few of the important morphological and agronomic attributes of these varieties, including some which might have a bearing on survival in mixtures, are presented in table 1.

Twenty grains of each of these fourteen varieties were mixed together and the mixture was dibbled in two rows in the field in 1943. At the end of the season, the number of plants available in each of these varieties was recorded. The plants were harvested as a bulk and in the next season about 4,000 plants were classified according to variety. In the later generations, a random sample of the previous year's harvest was drilled in 1/6 to 1/4 acre plots. Six plots, $6' \times 6'$, were marked out each year, at random, for the determination of the varietal make-up of the population. In the last season, 1947-48, data on the productivity of the surviving plants belonging to the different varieties were also recorded.

Experimental Results

The change in the varietal composition of the mixture was studied over five generations and at the end of the last season, the proportion of each variety in a random sample of the grains was also recorded. The data are summarised in table 2.

TABLE I

Morphological and agronomic characteristics of the wheat varieties included in the mixture

Species and variety	Chrom. No. (2n)	Early growth habit	Stature	Straw strength	Awning	Glume character	Grain size	Shedding	Maturity (Days to earing)	Remarks
T. aestivum										
N.P. 4 ..	42	Erect	Tall	Strong	Awnless	White pubescent	Bold	Non-shedding	Early (76–90)	Rust escaping; cultivated.
N.P. 114 ..	42	Erect	Midtall	Strong	Fully bearded	Brown Pub.	Med.	Non-shedding	Midearly (91-105)	Rust susceptible, cultivated.
T. sphaerococcum										
E. 14 ..	42	Erect	Dwarf	Strong	Fully bearded	White glabrous	Small	Non-shedding	Early (76–90)	Rust escaping; cultivated.
T. polonicum										
E. 17 ..	28	Spreading	Tall	Med. strong	Fully bearded	White glabrous long	Long and slender	Non-shedding	Late (121-135)	Moderately susceptible to rust; Exotic, cultivated.
T. durum										
E. 16 ..	28	Sltly-spread.	Tall	Strong	Fully bearded	Brown glab.	Bold	Non-shedding	Late (121-135)	Mod. susc. to black rust, cultivated.
S. 40	28	Sltly-spread.	Tall	Strong	Fully bearded	White Pub.	Bold	Non-shedding	Late (121-135)	Susc. to Bl. rust; cultivated.
T. dicoccum										
E. 56	28	Erect	Mid-dwarf	Strong	Fully bearded	White glab.	Med.	Fragile rachis	Midearly (91-105)	Susc. to yellow and brown rust; cultivated.
T. dicoccoides										
E. 303 ..	28	Spreading	Dwarf	Med. Strong	Fully bearded	Brown glab.	Long slender	Fragile rachis	Late (121-135)	Mod. susc. to Bl. and Br. rust; wild.

TABLE I—*Contd.*

Species and variety	Chrom. No. (2n)	Early growth habit	Stature	Straw strength	Awning	Glume character	Grain size	Shedding	Maturity (Days to earning)	Remarks
T. pyramidale										
E. 228 ..	28	Erect	Medium	Strong	Fully bearded	White pub.	Bold	Non-shedding	Midlate (106-120)	Mod. susc. to Bl. rust; wild; broad leaves.
E. 229 ..	28	Erect	Medium	Strong	Fully bearded	White glab.	Bold	Non-shedding	Midlate (106-120)	,, ,,
T. persicum										
E. 18 ..	28	Sltly. spread.	Tall	Med. str.	Fully bearded	Black pub.	Med.	Non-shedding	V. late (136-150)	Mod. susceptible to rusts; wild
T. timopheevi										
E. 79 ..	28	Spread.	Dwarf	Strong	Fully bearded	White glab.	Med. shrivelled	Non-shedding	Extremely late (151-165)	Rust resistant; wild.
T. monococcum										
E. 25 ..	14	Spread.	Dwarf	Strong	Fully bearded	White glab.	Small shrivelled	Fragile Rachis	Extremely late (151-165)	Rust resistant; Exotic cultivated.
T. aegilopoides										
E. 302 ..	14	Spread.	Dwarf	Med. Strong	Fully bearded	Brown glab.	Midlong shrivelled	Fragile Rachis	Extremely late	Mod. resistant to rust; wild.

TABLE 2

Varietal composition of a mixture of fourteen wheat varieties in different generations of bulk handling

Species and Variety		Proportion in original mixture (%)	Proportion of plants (%) in bulk generation					Proportion of grains at the end of the V bulk generation (1948)
			I 43–44	II 44–45	III 45–46*	IV 46–47	V 47–48	
T. aestivum	N.P. 4	7·1	7·5	21·1	..	81·1	84·7	94·40
,,	N.P. 114	7·1	9·5	18·7	..	5·4	5·3	2·10
T. sphaerococcum ..	E. 14	7·1	7·5	7·9	..	3·6	3·1	1·13
T. pyramidale	E. 229	7·1	6·8	15·2	..	5·6	4·0	1·15
,,	E. 228	7·1	8·0	10·2	..	2·5	0·5	0·20
T. persicum	E. 18	7·1	6·8	10·5	..	1·6	0·5	0·14
T. durum ..	S. 40	7·1	10·2	8·8	..	0·0	0·0	0·00
	E. 16	7·1	10·2	4·7	..	0·0	0·0	0·00
T. polonicum	E. 17	7·1	8·0	0·6	..	0·0	0·0	0·00
T. dicoccum	E. 56	7·1	1·4	2·3	..	0·0	0·0	0·00
T. dicoccoides	E. 303	7·1	3·4	0·0	..	0·0	0·0	0·00
T. timopheevi	E. 79	7·1	9·5	0·0	..	0·0	0·0	0·00
T. monococcum	E. 25	7·1	6·0	0·0	..	0·0	0·0	0·00
T. aegilopoides	E. 302	7·1	4·8	0·0	..	0·0	0·0	0·00
T. aestivum hybrids†		0·0	0·0	0·0	..	0·4	1·7	0·98

*Observations were not recorded in this season.

†Probably intervarietal crosses between N.P. 4 and N.P. 114. Four recombinant types were noted: I. Awnletted; brown, pubescent glume. II. Awnletted; brown, glabrous glume. III. Half-bearded; white, pubescent glume. IV. Fully bearded; white, pubescent glume.

The proportion of N.P. 4 in the mixture showed a consistent increase in all the generations, from 7·5 per cent at the beginning to 94·4 per cent at the end of the experiment. Other varieties, like N.P. 114 and E. 229, increased in proportion for two or three generations but started to fall off later. Some, like E. 16, E. 17 and E. 56, began to decrease in the second generation itself, while others were completely absent even in this generation. This differential behaviour of the varieties is clearly brought out by the survival curves in Fig. 1, where, in order to avoid confusion, only one variety of each of the above groups is represented. It is seen from Table 2 that at the end of the experiment only N.P. 4 and N.P. 114 (*T. aestivum*, 2n-42), E. 14 (*T. sphaerococcum*, 2n-42), E. 18 (*T. persicum*, 2n-28) and E. 228 and E. 229 (*T. pyramidale*, 2n-28) were present in the population, N.P. 4 being the dominant variety.

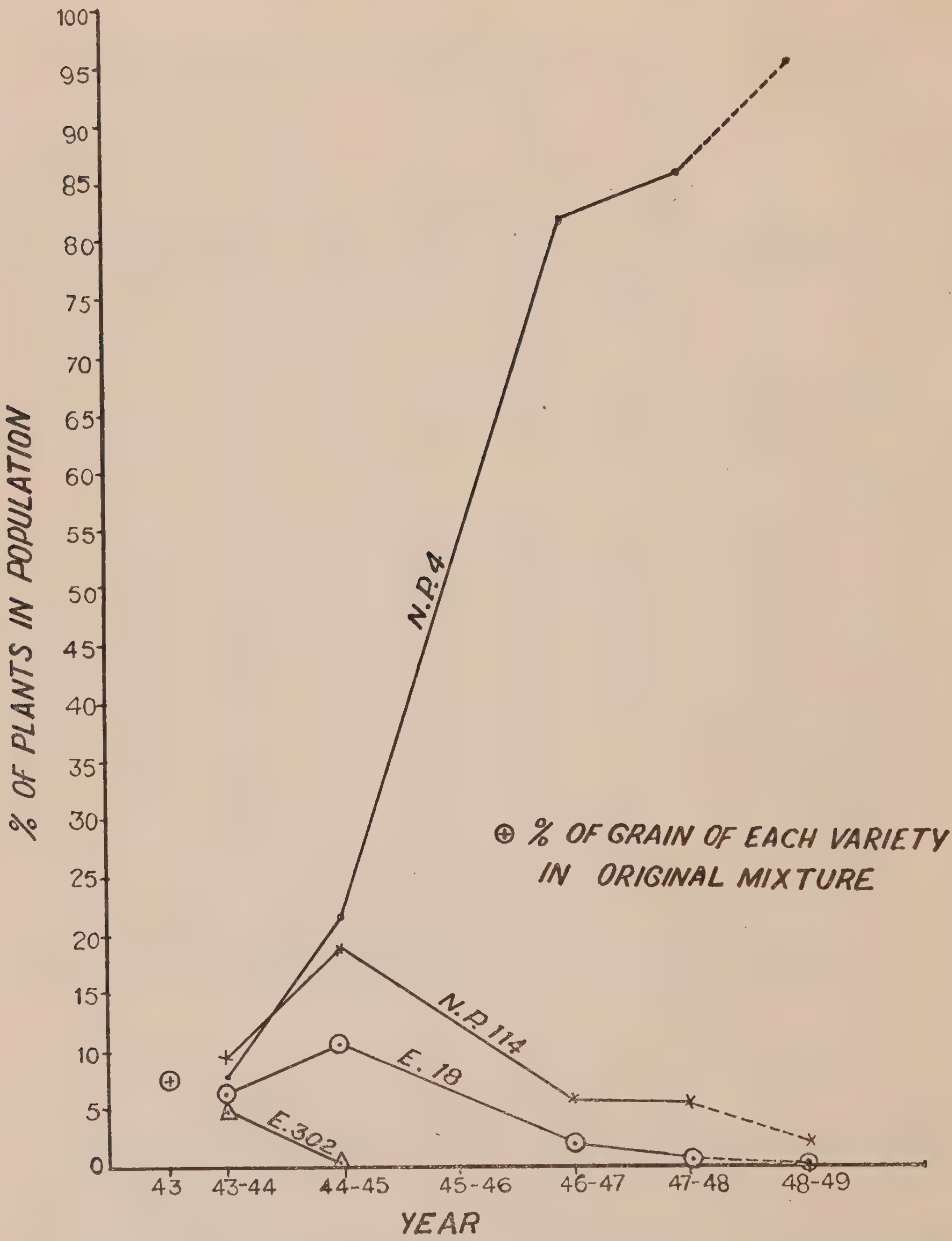

Fig. 1. Percentage of plants of four varieties in the mixture in successive generations of bulk handling. The value for 1948-49 is derived from the percentage of grains of each variety present in random samples of the grain of the 1948 harvest.

Association between morphological and physiological factors and survival

It has been suggested in the past by various workers that diverse factors such as early growth habit, maturity, grain shedding, grain size etc., may be important in determining the survival ability of a variety. The data in regard to some of these characters have been included in table 1 and the association existing between these and survival percentage is briefly considered below:

Maturity:

This may be measured as the number of days taken from sowing to ear emergence. Observations recorded in pure stands of these varieties during two seasons, 1947-48 and 1948-49, are presented in Fig. 2, where the proportion of the different varieties in the mixture, after one and four generations of bulk-growing, are also indicated. There appears to be a general tendency for late varieites to get eliminated from the population at a faster rate. Thus, for instance, the very late varieties, E. 302, E. 303, E. 25 and E. 79 are almost or completely absent from the second generation itself. This is perhaps to be expected from the fact that these varieties can be maintained at Delhi, even in pure stands, only if they are subjected to appropriate photoperiodic

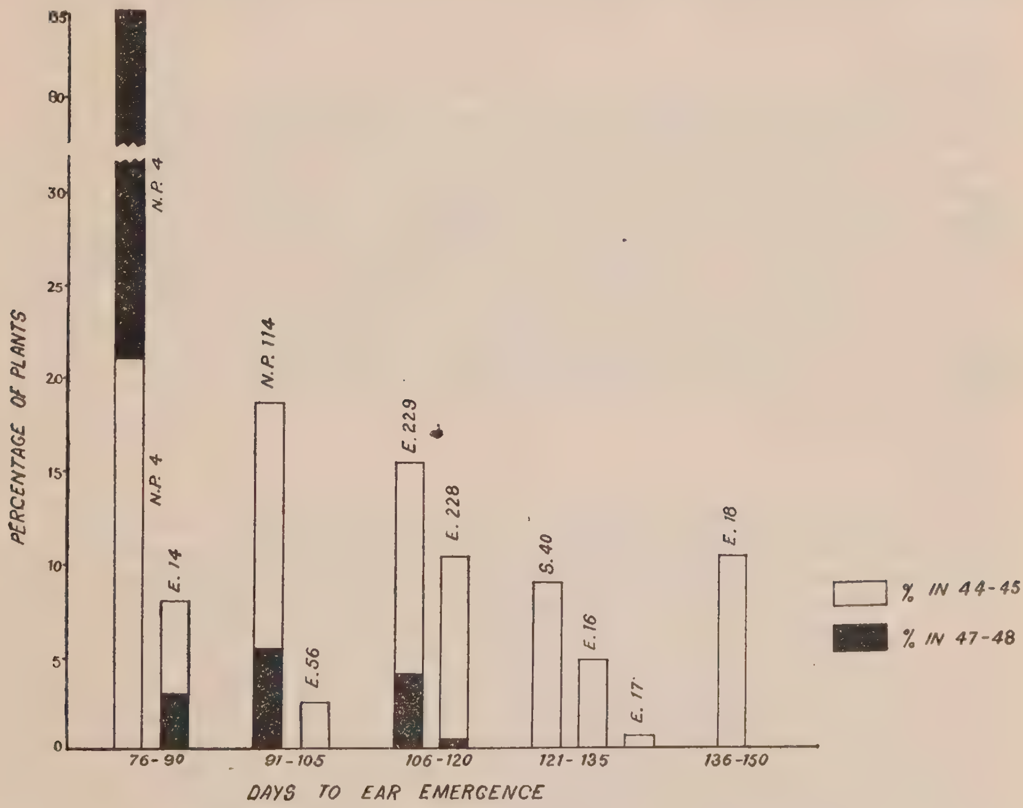

Fig. 2. Relationship between the time taken for ear emergence and the percentage proportion, in the mixture, of different varieties after one and four generations of bulk handling. Varieties which were absent from the first generation of competition [E. 303 (121-135 days) and E. 25, E. 79 and E. 302 (151-165 days)] are not represented.

treatment. The relatively slower elimination of E. 18, another very late variety, however, suggests that maturity is not the only factor determining survival in the mixed population under study.

Early growth habit:

An erect growth habit, it has been held, may enable such varieties to emerge successfully in competition with spreading varieties. The data presented in tables 1 and 2 show, however, that some erect varieties have been eliminated equally with the spreading types.

Grain size:

It has also been suggested that plants raised from bold grains yield better than those derived from small grains, presumably because their greater initial capital results in an increased rate of growth in the earlier stages. In the present case, however, long and slender grained varieties as well as plump grained *durums* and *persicums* have been equally eliminated and it would, therefore, appear that boldness of grain by itself has no special advantage so far as competing ability is concerned, atleast at the inter-specific level. Even within the species *T. aestivum*, the more competitive variety, N.P. 4, has slightly smaller grains than the less competitive variety, N.P. 114.

Grain Shedding:

While this factor may not have any adverse effect on survival in natural populations, in the conditions of the present experiment varieties which shed their grain at maturity are likely to be under-represented in the random sample of grain taken for sowing the succeeding year's crop. It is doubtful, however, whether this factor has operated to any great extent to bring about the observed changes since the majority of the varieties with fragile rachis were also very late. The sharp fall, from the 2nd to the 4th generation, in the one early variety with fragile rachis, E. 56, may perhaps have been partly due to this cause, though loss through random fixation, inherent in the smallness of samples, cannot be entirely excluded. It may also be noted that in N.P. 114, the glumes do not hold the grain firmly after they have ripened, so that considerable loss of grain occurs, especially if the crop is harvested after it is dead ripe (Pal, 1944). Such grain loss might have contributed to the sharp fall, in one or two generations, in the proportion of this variety, from 18·7 per cent (in 44-45) to 5·4 per cent (in 46-47).

Reproductive ability:

In the ultimate analysis, survival capacity must be directly related to two factors, i.e., the number of grains produced per plant and the successful establishment and normal reproduction of the plants arising from these grains. All the other factors which influence survival rate, can do so only through one of these aspects.

The overall-percentage of plants which survived to the reproductive stage in the first generation was 49 per cent in the present study. Except for one or two varieties, there was no marked varietal differences in establishment capacity. This absence of any large varietal differences in seedling mortality, which has also been observed in another experiment, suggests that such differences may not play an important role in the domination of one variety over the others in the mixture. An exception to this is perhaps provided by the shrivelled seeds produced by the extremely late varieties. It is possible that such shrivelled seeds may have been completely, or to a large extent, inviable.

The other component of reproductive ability, i.e., the relative productivity of the different varieties can itself be split into two subcomponents—the number of ears per

plant and the number of grains per ear. An estimate of these factors was obtained for the surviving varieties in 1947-48, when the experiment was terminated. In this season, all the plants in the sample plots were carefully uprooted, classified according to variety and scored for these characters. The data are summarised in table 3.

TABLE 3

Reproductive ability of four surviving varieties in the fifth generation of bulk handling of the mixture

Species and variety		Ears/plant	Average No. of grains/ear	Grains per plant	Proportion% in 47-48	
					Plants	Grains
T. aestivum ..	N.P. 4	5·3	31·4	108·2	84·73	94·38
	N.P. 114	2·8	19·9	55·1	5·33	2·02
T. pyramidale ..	E. 229	1·4	32·2	54·8	3·90	1·15
T. sphaerococcum	E. 14	2·0	27·4	54·8	3·12	1·13

It appears that the survival rate of these varieties follows quite closely their reproductive ability. Thus, the dominant variety, N.P. 4, shows overwhelming superiority with regard to both the subcomponents, ears per plant and grains per ear.

N.P. 114 occurs more frequently than the other two varieties, persumably because of the greater number of ear bearing tillers per plant in this variety, though it is inferior to E. 229 and E. 14 in the number of grains produced per ear. In respect of the number of grains produced per plant, these three varieties seem to be at par. However, since ear number per plant is highly susceptible to environmental factors, N.P. 114 could conceivably, loose its advantage in some seasons. E. 229 on the other hand, may owe its survival to the fact that it produces a large number of grains per ear. Attention should, therefore, be paid to both these subcomponents of relative productivity in assessing the competing ability of varieties.

DISCUSSION

Due to various reasons, the present experiment had to be terminated within a rather short time, as such experiments go. Also, some of the varieties included in the experiment could only be maintained with difficulty even in pure stands and as such might have served to give a distorted picture of the behaviour of some of the other varieties. However, even though the experiment is by no means complete, a few tentative conclusions can be drawn from the results obtained.

So far as can be gathered from these observations, no constant association could be demonstrated between the various morphological characters studied and the surviving ability of the different varieties. Extremely late varieties, however, do tend to get eliminated fast, perhaps due to their inability to produce viable seeds under the unfavourable climatic conditions obtaining at the time of their flowering. It may also be possible that some morphological factor not studied in the present experiment, such as root development, may be responsible for the differential rate of survival.

Another factor, which may have been responsible for the quicker elimination of some of the components of the mixture, is the occurrence of disease epidemics.

1945-46 and 1946-47 were seasons when rust was present in an epidemic form and it is quite likely that rust susceptible varieties, like E. 228, E. 18 and N.P. 114, might have produced a greater proportion of shrivelled, less-viable seeds than early, rust escaping varieties like N.P. 4 and E. 14. This factor could have been responsible for the complete elimination of susceptible varieties like S. 40, E. 16 and E. 17 within one generation. It is, however, interesting to note that the epidemic of 46-47 has not resulted in any marked reduction in the frequency of N.P. 114 in 1947-48, while the frequency of E. 228 and E. 18, shows a comparatively sharp fall in the same season. This might, perhaps, be because of the fact that N.P. 114 has some measure of adult resistance to black rust. Since no data were recorded in 45-46, it is not possible to evaluate the effect of this season's epidemic on the frequency of occurrence of N.P. 114.

Sakai (1955) has shown that, in a number of autogamous plant species, when individual plants of a less competitive variety are surrounded with individuals of a more competitive variety, the former suffer considerable decrease in a number of characters including some components of yield like plant weight, number of inflorescences, number of seeds per plant, weight of seeds etc. Thus, for example, the number of seeds per plant produced by an improved variety of *japonica* rice in pure culture was more than the number produced when this variety was competing against wild 'red rice'. In the present experiment, especially in the later generations, the proportion of N.P. 4 is so large that it would not be far wrong, if each plant of the less frequent varieties is visualized as being more or less completely surrounded by N.P. 4 plants and competing with them. It would, therefore, be interesting to compare the productivity components of these varieties in pure stands with those presented in table 3, which record their performance under competition with N.P. 4. Unfortunately, data for N.P. 114 only is available and that too from a different experiment carried out under different conditions of planting, though at the same location and over the same years. In Fig. 3, the data with regard to ear number per plant, grain number per ear (calculated on the assumption that the two varieties produced approximately the same number of grains per spikelet) and the number of grains per plant in pure stands are compared with those recorded for the same characters under competition. It is seen that while N.P. 114 is at least equal, if not superior, to N.P. 4 in pure stands, it is considerably inferior in mixed stands. While no doubt a great deal of caution must be exercised in drawing conclusions from a comparison of results from these two different experiments, taken together, it does appear that N.P. 114 is less competitive, in the sense of Sakai, than N.P. 4. This, however, needs further study on the lines so clearly set out by this author.

It can hardly be doubted that intergenotypic competition has played an important role in natural selection. The results recorded here have shown that there exists certain variability with regard to competitive ability, i.e. ability to survive in mixtures, even among varieties within a species. It would appear reasonable to assume that gene mutations conferring greater competitive ability do occur in natural populations and that selection of such mutant types would result in the improvement of the competitive ability, in that environmental niche, of the population as a whole. Such an increase in competitive ability has been shown to occur even in laboratory populations of *Drosophila* within comparatively short periods (Moore, 1952). The fact that N.P. 4 is more competitive than N.P. 114 could then, perhaps, be attributed to the fact that the former was a selection from a natural indigenous population while the later arose from a chance hybridisation in an introduced Australian variety, which had not undergone extensive natural selection under Indian conditions.

Competitive ability, then, is likely to be a valuable agronomical character since highly competitive strains may not suffer as much as the less-competitive ones from weed competition. Further, in countries like India, where improved seeds very often get mixed up with local types, improved varieties lacking competitive ability might

be quickly replaced by the local types in the mixtures so that there may be an apparent deterioration or running out of the variety. Such lack of competitive ability might perhaps be one reason why, even in advanced countries, apparently superior varieties, which give consistently high yields in experimental plots are not accepted by farmers who continue to grow varieties which give lower yields in pure stands but, probably, have greater competitive ability and high survival value in mixtures.

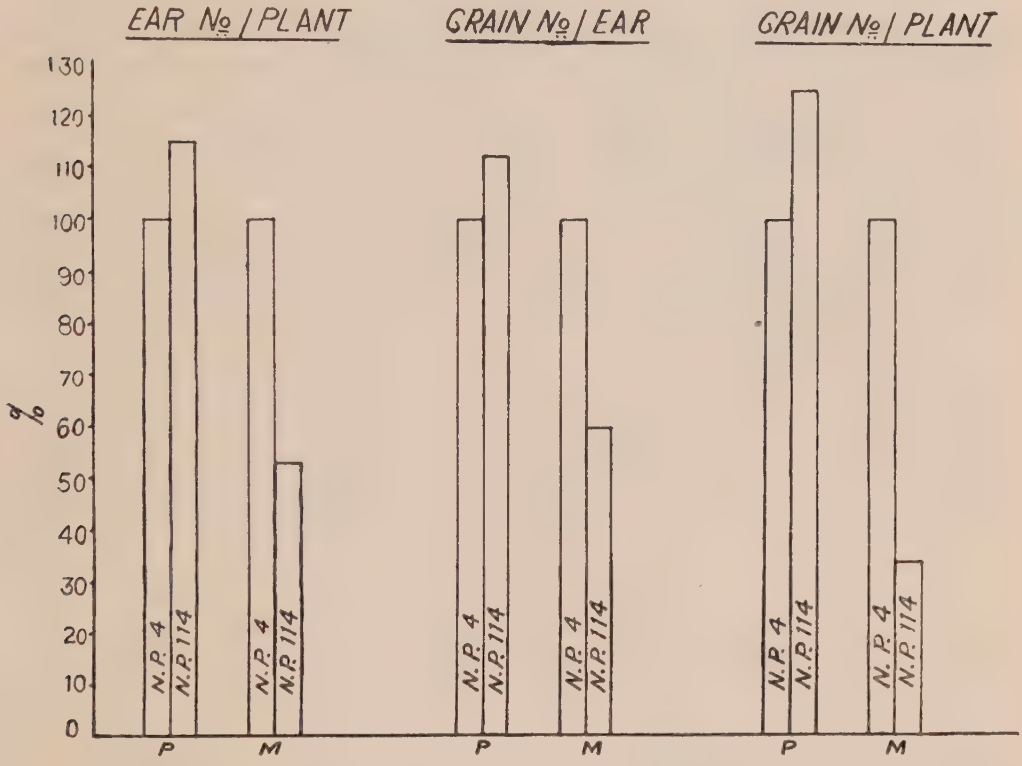

Fig. 3. The performance of N.P. 114 relative to N.P. 4 (=100 per cent) with regard to three contributing characters, when grown in pure stands and in the fifth generation of bulk growing (P=value in pure stands; M=value in mixed stands). See text for discussion.

Summary

Intergenotypic competition over six years among fourteen varieties of wheat, belonging to eleven species, as measured by percentage of survival in successive generations of mixed growing, has been studied. No consistent association existed between survival ability and a number of morphological and agronomic characters. The main factor which determined the degree of survival of any variety appeared to be its reproductive ability, i.e., its relative productivity and the capacity of the seeds so produced to reach the reproductive stage in the succeeding generation. No marked varietal differences were, however, noted with regard to mortality in the pre-reproductive stage. Rust epidemics occurred during two of the experimental years and could have been partly responsible for the sharp fall in the frequency, or complete elimination, of susceptible varieties within one or two generations. It has been suggested that less

competitive varieties may suffer a decrease with regard to some of the components of productivity when grown in a mixture with more competitive varieties and, therefore, have less survival value in such mixtures. The possible agronomic importance of greater competitive ability has been pointed out.

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STUDIES ON WORLD GENETIC STOCK OF RICE: I. PATTERNS OF ANTHOCYANIN PIGMENT DISTRIBUTION

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Presence or absence of anthocyanin pigmentation in different plant organs is one of the most important characteristics in rice and has been used by workers for classification of varieties. Occasional association between genes for pigment in particular parts with those influencing such characters like spikelet sterility, vigour and productivity has been recorded (Ramiah, 1945).

Classification of varieties according to the occurrence of pigmentation in definite patterns has been done by Hector (1916, 1922), Chao (1928), Jones (1929), Nagamatu (1943), Ramiah (1945) and Dave (1949). In the present paper, the pigmentation pattern in the World collection of genetic material of rice maintained at this institute has been reported.

Material and Method

Out of 2572 varieties, belonging to 30 different countries, 796 types that have been found to be pigmented were studied for pigmentation pattern in fifteen parts viz., leaf sheath, leaf blade, leaf tip and margin, auricle, ligule, junctura, junctura back, internode, node, septum, pulvinus, glume, lemma-palea, apiculus and stigma. Awn colour has not been included, as its colour is mostly the same as that of the apiculus. The difference in the intensity of pigmentation has not been taken into consideration. Table 1 gives the number of pigmented varieties studied from each country.

Table 1

Country	No. of varieties	Country	No. of varieties
			712
1. Africa ..	41	16. Iraq	3
2. Argentina ..	4	17. Italy	5
3. Australia ..	8	18. Japan ..	15
4. Brazil	5	19. Malaya ..	6
5. Bulgaria ..	1	20. New Guinea ..	2
6. Burma ..	20	21. Netherlands ..	1
7. Ceylon.. ..	19	22. Pakistan ..	11
8. China	28	23. Peru	2
9. Costa Rica ..	3	24. Philippines ..	6
10. Egypt	3	25. Portugal ..	3
11. Fiji	3	26. Russia ..	5
12. Hongkong ..	1	27. Thailand ..	4
13. India	545	28. Taiwan ..	1
14. Indo-China ..	14	29. U.S.A. ..	10
15. Indonesia ..	17	30. Vietnam ..	10
	712	Total	796

The varieties have been classified into groups, varieties showing pigmentation in an equal number of parts being placed in the same group. The group with one part pigmented is designated I, that with two parts pigmented II, that with three parts pigmented designated III and so on. Within each such group a number of different classes are designated as I-A, I-B, II-A, II-B, II-C and so on, each class containing varieties with identical plant parts pigmented, as shown in table 2.

Table 2

Number of rice varieties in different pigmentation pattern classes

\+ Presence of pigments
— absence of pigment

	Groups	Leaf sheath	Leaf blade	Leaf tip & margin	Auricke	Ligule	Junctura	Junctura-back	Internode	Node	Pulvinus	Septum	Glume	Lemma-palea	Apiculus	Stigma	Total
I	A	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	30
	B	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	8
II	A	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	19
	B	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	44
	C	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	5
III	A	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	279
	B	+	—	—	—	—	—	—	—	—	—	—	+	—	+	—	25
	C	+	—	—	—	—	—	—	—	—	—	+	—	—	+	—	4
	D	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—	11
IV	A	+	—	—	—	—	—	—	+	—	—	—	—	—	+	+	53
	B	+	—	—	—	—	—	—	—	—	+	—	—	—	+	+	4
	C	+	—	—	—	—	—	—	—	—	—	+	—	—	+	+	33
	D	+	—	—	—	—	—	—	—	—	—	—	+	—	+	+	8
	E	+	—	—	—	—	—	—	—	—	—	—	+	+	+	—	7
	F	+	—	—	—	—	—	—	—	—	—	+	+	—	+	—	12
	G	+	—	—	+	—	—	—	—	—	—	—	—	—	+	+	6
	H	+	—	—	+	—	—	—	—	—	—	—	+	—	+	—	2
V	A	+	—	—	+	+	—	—	—	—	—	—	—	—	+	+	6
	B	+	—	—	+	—	+	—	—	—	—	—	—	—	+	+	28
	C	+	—	—	—	—	—	—	+	—	—	+	—	—	+	+	5
	D	+	—	—	—	—	—	—	—	—	+	+	—	—	+	+	2
	E	+	—	—	+	—	—	—	+	—	—	—	—	—	+	+	3
	F	+	—	—	—	—	—	—	—	—	—	+	+	+	+	—	2
	G	+	—	—	—	—	—	—	+	+	—	—	—	—	+	+	2

Table 2—(*Contd.*)

	Groups	Leaf sheath	Leaf blade	Leaf tip & margin	Auricke	Ligule	Junctura	Junctura-back	Internode	Node	Pulvinus	Septum	Glume	Lemma-palea	Apiculus	Stigma	Total
	H	+	−	−	−	−	−	−	+	−	−	+	+	−	+	−	3
	I	+	−	−	−	−	−	−	+	−	+	−	−	−	+	+	2
	J	+	−	−	+	+	−	−	−	−	−	−	+	−	+	−	2
	K	+	−	−	+	−	+	−	−	−	−	−	+	−	+	−	2
VI	A	+	−	−	−	−	−	−	+	−	+	+	−	−	+	+	2
	B	+	−	−	+	+	+	−	−	−	−	−	−	−	+	+	18
	C	+	−	−	+	−	+	−	+	−	−	−	−	−	+	+	9
	D	+	−	−	+	−	+	−	−	−	+	−	−	−	+	+	6
	E	+	−	−	+	−	+	−	−	−	−	+	−	−	+	+	11
	F	+	−	−	+	−	+	+	−	−	−	−	−	−	+	+	7
	G	+	−	−	−	−	−	−	+	−	−	−	+	+	+	+	2
	H	+	+	+	−	−	−	−	−	−	−	+	−	−	+	+	2
VII	A	+	−	−	+	+	+	−	−	−	−	+	−	−	+	+	2
	B	+	−	−	+	+	+	−	+	−	−	−	−	−	+	+	4
	C	+	−	−	+	+	+	−	−	−	+	−	−	−	+	+	2
	D	+	−	−	+	−	+	−	−	−	+	+	−	−	+	+	4
	E	+	−	−	+	−	+	+	+	−	−	−	−	−	+	+	3
	F	+	−	−	+	−	+	−	+	−	+	−	−	−	+	+	2
VIII	A	+	−	−	+	+	+	−	+	−	+	−	−	−	+	+	4
	B	+	−	−	+	+	+	−	+	−	−	+	−	−	+	+	3
	C	+	−	−	+	+	+	−	−	−	−	+	+	−	+	+	2
	D	+	−	−	+	+	+	+	+	−	−	−	−	−	+	+	2
	E	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	2
	F	+	−	−	+	+	+	−	+	−	−	−	+	−	+	+	3
	G	+	+	+	+	+	−	+	−	−	−	−	−	−	+	+	4
	H	+	−	−	+	−	+	+	+	−	+	−	−	−	+	+	2
IX	A	+	−	−	+	−	+	−	+	−	+	+	+	−	+	+	2
	B	+	+	+	+	+	−	+	−	−	−	−	+	−	+	+	3
	C	+	−	−	+	+	+	+	+	−	+	−	+	−	+	−	2
	D	+	+	+	+	+	−	+	−	−	−	−	+	−	+	+	2
X	A	+	+	+	+	+	+	+	−	−	−	−	+	−	+	+	2
XI	A	+	−	−	+	+	+	+	+	−	+	−	+	+	+	+	2
XII	A	+	−	+	+	+	+	+	+	+	−	+	+	+	+	−	3
																	719

Observations:

Out of 796 pigmented varieties, 719 could be broadly grouped into 57 classes; frequency in each of the different classes is indicated in table 2. The remaining 77 varieties did not fall into any particular class, each one forming a different class by itself; these have been excluded from the table.

The most common pigmentation patterns are five, namely

(i) Pigmentation in leaf sheath, apiculus and stigma (279)
(ii) Pigmentation in leaf sheath, apiculus, stigma and internode (53)
(iii) Pigmentation in leaf sheath and apiculus (44)
(iv) Pigmentation in leaf sheath, apiculus, stigma and septum (33).
(v) Pigmentation in apiculus only (30).

In 55 out of the 57 classes, the apiculus is pigmented; the leaf sheath shows pigmentation in 53 of the classes while 43 have pigmented stigma. Pigmentation in the leaf sheath, apiculus and stigma is present in 40 of the 57 classes.

Discussion

The study of the inheritance of pigmentation patterns is complicated as expression of anthocyanin pigmentation in various plant parts (both quality and intensity) is considerably affected by various environmental factors [(Jones 1921) and Ramiah (1945)]. But in the present study, as intensity of pigmentation is not taken into consideration in the determination of the pattern, such a difficulty was not experienced.

No case has been met with where all the plant parts are pigmented. Even in cases where the whole plant looks deeply pigmented, one or more of the following parts are not pigmented, viz., internode, pulvinus, septum and leaf blade. With the exception of Nagamatu (1943), other workers have also reported similar findings. It may, however, be mentioned that observations made by Nagamatu were confined to ten parts only and did not include septum and pulvinus.

Where only one plant part is pigmented, it is either the apiculus or the stigma. If two parts are pigmented, it may be one of the following three combinations: II A, II B and II C. Based on the frequency of the different classes in table 2, it can be concluded that the gene for pigmentation in the leaf sheath is closely linked with that for pigmentation in apiculus and stigma, the linkage between leaf sheath and apiculus being relatively closer than that between leaf sheath and stigma. The above findings are in agreement with those of Jones (1929), Nagamatu (1943), and Dave (1949).

The types with no pigment in apiculus but with pigment elsewhere are very few in number. A similar observation was also made by Ramiah (1945). This would naturally raise the doubt whether the gene for pigmentation in apiculus is essential for expression of pigmentation in other parts (Nagao, 1951). Although no definite conclusion may be possible with regard to this point among the *japonicas* (which number only 15 in the present study), it is reasonable to conclude that apiculus colour gene does not seem to be absolutely essential for pigmentation in other parts at least in the *indicas*. If the contention of Nagao is accepted, it would be necessary to postulate the existence of several inhibitory factors, other than those already known to suppress the pigmentation in leaf blade and stigma.

The preponderance of rice varieties with green leaf-blade observed in the present study and also recorded by Jones (1929), Nagamatu (1943), and Ramiah (1945) is probably due to the existence of inhibitory gene suppressing the expression of the gene for pigmentation, which otherwise is dominant.

Of particular interest is the occurrence of pigmentation in the stigma. Normally, pigmentation in the stigma is present when colour is observed in any of the following three sets of parts:

(i) internode, apiculus and leaf sheath.

(ii) septum, apiculus and leaf sheath.

(iii) lemma and palea, apiculus (deeply spreading) and leaf sheath.

However, when the glume is also pigmented in addition to the above-mentioned parts, there has been a tendency for the stigma to be white. The absence of pigmentation in the stigma under such conditions may be due to the action of inhibitory gene or genes associated with the gene or genes governing pigmentation in the glume. The interrelationship of such inhibitory gene or genes remains to be studied.

Another pattern in anthocyanin pigmentation is the nature of diffusion of the colour from the apiculus down the lemma-palea. Varieties exist with varying degrees of lemmapalea pigmentation, ranging continuously from a slight tinge in the apiculus at one extreme to deep colouration at the other extreme. Takahashi (1957) explains the occurrence of pigmentation in the apiculus and the differences in the colour intensity as being due to genes present in three loci, **C, Sp** and **A** with the presence of multiple allelic systems at the **C** and **Sp** loci. No explanation has so far been offered for the differential diffusion of the pigment formed at the apiculus. Studies carried out at this institute suggest the possibility of another allelic system present in a different locus (besides the **C, Sp** and **A**) being responsible for differences in the pigment diffusion.

Summary

Sevenhundred and ninetysix pigmented rice varieties from 30 countries were studied for anthocyanin pigmentation pattern in fifteen plant parts. Of these, 719 types were grouped into fifty seven classes; the remaining seventyseven types could not be grouped, as each one formed a different class by itself. There was no variety in which all plant parts under study were pigmented. Certain inferences have been drawn on the inter-relationship of genes and gene loci governing pigmentation in different plant parts.

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STARCH GRAIN CHARACTERISTICS AND AMYLOSE CONTENT IN RELATION TO POLLINATION TIME AND SEED SIZE, AND GERMINATION BEHAVIOUR IN *PHASEOLUS* SPECIES[1]

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The starch of beans is often considered a single substance made up of two components, amylose and amylopectin. Amylose content is considered to be a more important character than amylopectin content, because of its industrial uses in the manufacture of edible casings, in paper industry as a binding agent, and in textile manufacture as a permanent finish where other hydroxylated polymers have been used (Senti, 1958).

Two types of germination (epigeal and hypogeal) are commonly found in the plant kingdom. In the genus *Phaseolus*, the common type of germination is of the epigeal type. However, the hypogeal type of germination is also found in some wild-growing species like *P. polystachyus* L. It is known that, under compact soil conditions, many seedlings with a heavy load in the cotyledons cannot come out of the ground, and that this reduces the percentage of emergence. It should be profitable, therefore, if the characteristic of hypogeal germination could be transferred from wild *Phaseolus* species to the cultivated types.

This paper presents the observations on starch grain characteristics (size, shape, hylum (hilum)[3] and lamella development), and the amount of amylose in relation to pollination time and size of beans in certain varieties of *P. lunatus* L., *P. polystachyus* L. and in the F_1 of the cross (*P. lunatus* L var. Concentrated Fordhook × *P. polystachyus* L.). Studies were also conducted on the germination behaviour of *P. lunatus* L. var. Concentrated Fordhook, *P. polystachyus* L. and the F_1 of the cross (*P. lunatus* L. var. concentrated Fordhook X *P. polystachyus* L.).

Material and Methods

Five varieties of lima beans, namely Burpee Bush, Clark's Bush, Utah 16, Wasatch Bush, and Concentrated Fordhook, belonging to the species *P. lunatus* L., were planted at the Farmington Field Station of the Utah State Agricultural Experiment Station, Farmington, Utah, on June 1, 1958. The plots were 22 feet long and 1½ feet wide. The crop growth was satisfactory. Weeding and irrigation was done at an interval of seven days throughout the growing season.

The germination studies were conducted in the greenhouse, and photographs were taken to show the relative location of cotyledons on seedlings of both parents and the F_1.

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[1] Contribution from the Utah State Agricultural Experiment Station, Logan, Utah, and from the Florida State Agricultural Experiment Station, Gainesville, Florida.

[2] This paper is based upon a dissertation submitted by the senior author to the Faculty of Graduate School of Utah State University in candidacy for the Degree of Master of Science (1959).

[3] According to many botany texts, the word hilum means the mark at the point of attachment of an ovule to its funicle. In addition, the word hilum has another meaning, i.e., the nucleus of a starch grain. To differentiate the meanings of the same word, Salunkhe and Pollard (1955a, 1955b) coined a word, "hylum" to mean the latter. In this article, hereafter, the word hylum (plural - hyla) will be used to designate the nucleus of a starch grain.

Sixty-six days after seeding, opened flowers of each variety were tagged (August 6, 1958). Flowers which had opened before noon on a particular date were tagged. Similarly, at seven day intervals, the flowers were tagged, on August 13, and August 20, 1958. The beans of the species *P. polystachyus* L. and of the F_1 of the cross *P. lunatus* × *P. polystachyus* were not tagged at these dates of pollination.

All the pods formed were harvested at full maturity on October 10, 1958. The harvested beans from each date of pollination were classified, by visual observation, into small, medium, and large groups. In each class five beans were selected. They were then dried at 158°F. for 24 hours. Each bean was weighed. In addition to this, the beans from species *P. polystachyus* were also classified into three groups, as mentioned above, with 5 beans in each group regardless of dates of pollinations. Since seeds of this species were very small, all five beans were weighed together in each group. As the supply of beans in the species cross was limited, only one bean was taken at random from 10 beans for study.

Dried and weighed beans were individually ground with a mortar and pestle. The ground material with a drop of distilled water on a slide was studied under the microscope for size, shape, hylum, and lamella development of starch grains with the combination of an eyepiece 10X and an objective 43X. Shape was recorded as circular, oblong, and irregular; hylum, as unhylumed and highly hylumed and lamella, as unlamellated and lamellated. In each microscopic field of a slide only one starch grain was considered as a part of the observation. In total, 45 seeds and 4500 starch grains in each variety were examined for the specific characteristics mentioned above. The statistical analysis of the data revealed that eight starch grains per slide and four slides per bean were satisfactory to reproduce rather identical results. Amylose content was determined according to the procedure standardized by McCready and co-workers (1950).

The data were analyzed statistically following the methods described by Ostle (1956).

Results and Discussion

The study was conducted to determine if there were differences in starch grain characteristics and amylose content for three size groups of beans from three subsequent pollination dates in species and varieties of genus *Phaseolus*. Germination studies were also made to ascertain the extent to which the hypogeal character had been transferred to the *lunatus* offspring. The results obtained during the course of the study are presented in table 1 and discussed below:

Starch grain

Burpee Bush: This is a large-seeded variety which grows well under Utah conditions. Table 1 shows that in general, weight of the beans and size of the starch grain varied from 1·38 to 0·62 grams and 34·82 to 21·46 microns respectively. Oblong, slightly hylumed, and unlamellated starch grains were present irrespective of the size groups of beans and dates of pollination. Unhylumed starch grains were rare in this variety, but slightly hylumed starch grains were commonly found. Salunkhe and Pollard (1955a) found that the maturity could be determined by examining the hylum of starch grains. They noted that as the maturity advanced the hylum of the starch grain began to thicken and ramify. Results obtained in this experiment did not agree with their results. Hence, it seems that the hylum development may be a varietal character.

Size of the starch grains was closely associated with weight and size of beans. Sharma (1956) observed a close association of size of starch grains with specific gravity

of potatoes. Table 1 shows that in three dates of pollination, the average size of starch grain was directly related to the size of bean. It can also be noted that with the advancement of the date of pollination the starch grain size decreased with respect to the weight and size of bean. This might be due to the differential availability of proper nutrition and heat units, where earlier-formed beans received more than the latter-formed beans.

TABLE 1

The effect of pollination time, size, and weight of beans on the size of starch grains of five lima bean varieties

		Burpee Bush		Clark's Bush		Utah 16		Wasatch Bush		Concentrated Fordhook	
Pollination time	Size of beans*	Wt. of seeds (gm.)	Size of starch grain (μ)	Wt. of seeds (gm.)	Size of starch grain (μ)	Wt. of seeds (gm.)	Size of starch grain (μ)	Wt. of beans (gm.)	Size of starch grain (μ)	Wt. of beans (gm.)	Size of starch grain (μ)
6–8–1958	L.	1·38	34·82	0·59	26·60	0·72	30·29	0·41	28·43	1·50	30·66
	M.	0·90	30·80	0·39	23·88	0·23	21·89	0·31	27·35	0·75	26·82
	S.	0·76	30·75	0·37	25·87	0·12	17·96	0·29	26·76	0·50	20·64
		** $\hat{y}=23{\cdot}34+8{\cdot}28X$		$\hat{y}=23{\cdot}50+3{\cdot}6X$		$\hat{y}=16{\cdot}13+20{\cdot}13X$		$\hat{y}=22{\cdot}34+15{\cdot}25X$		$\hat{y}=16{\cdot}16+6{\cdot}68X$	
13–8–1958	L.	1·24	35·23	0·54	25·06	0·57	28·34	0·43	27·59	1·47	31·40
	M.	0·78	29·64	0·39	22·85	0·31	24·08	0·28	26·58	0·78	27·25
	S.	0·62	27·64	0·28	19·30	0·21	19·01	0·20	22·23	0·60	23·93
		** $\hat{y}=20{\cdot}67+11{\cdot}68X$		$y=14{\cdot}49+19{\cdot}36X$		$\hat{y}=15{\cdot}12+24{\cdot}16X$		$\hat{y}=19{\cdot}19+20{\cdot}28X$		$\hat{y}=22{\cdot}03+6{\cdot}19X$	
20–8–1958	L.	1·23	31·29	0·47	23·81	0·46	26·57	0·37	26·74	1·50	30·66
	M.	0·80	24·26	0·28	22·34	0·30	24·88	0·28	24·96	0·75	26·82
	S.	0·68	21·46	0·24	21·43	0·21	21·27	0·24	22·53	0·50	21·96
		** $\hat{y}=9{\cdot}12+18{\cdot}34X$		$\hat{y}=18{\cdot}68+11{\cdot}66X$		$\hat{y}=16{\cdot}41+23{\cdot}75X$		$y=10{\cdot}05+49X$		$y=19{\cdot}16+17{\cdot}97X$	

* L=Large; M=Medium and S=Small.
** Regression of starch grain size (μ) on seed weight (gm.).

Utah 16: This selection has small beans with white cotyledons. It may be observed in table 1 that size and weight of beans and size of starch grains were smaller in this selection as compared to the variety Burpee Bush.

There was a direct relationship between weight and size of beans and size of starch grains. The pattern was similar for the three pollination dates. The shape, hylum, and lamella development of starch grains were also similar to the above mentioned variety.

Clark's Bush: This variety is small seeded with green cotyledons. Table 1 shows that, on the average, the size of starch grains was similar to Utah 16. Oblong starch grains were present more frequently than were the other types. Unhylumed starch grains were absent. Unlamellated starch grains were almost absent on the pollination date of August 6, 1958, but were present to the extent of 18 per cent on the pollination dates of August 13 and August 20, 1958. Frey-Wyssling (1953) suggested that the lamellae were due to the alternation of day and night, with the dense, highly refracting part of each lamella being deposited during the day. Van de Sande-Bakhuyzen (1926) showed that if external conditions were constant, lamellation did not occur, because nutritive material was then always available in the same concentration. On

the contrary, Roberts and Proctor (1954) stated that starch grains formed in potato tubers from plants grown under constant light and temperature showed lamellation, clearly indistinguishable from starch grains formed in tubers grown under normal field conditions. Keeping the above discussion in view, the conclusion could be drawn that the lamellae did occur in all starch grains but were not visible under the microscope, with the combination of 10X and 43X lenses, due to the lesser difference in the refractive index of starch deposited during the day and at night, in comparison to other varieties. The percentages of variation in starch grain sizes, which were accounted for by weight and size of beans, from the three pollination dates were 6·7 per cent, 62·8 per cent, and 38·5 per cent, respectively.

Wasatch Bush: This variety is small-seeded with green cotyledons. The variations in weight and size of beans, and size of starch grains were greater than in Clark's Bush. Oblong, highly hylumed, and lamellated starch grains were common as compared to other characteristics (Table 1). It was found that the hylum was greatly ramified and generally formed along the long axis of the starch grains, which conforms with the findings of Salunkhe and Pollard (1955b). However, in a few cases, the hylum was formed along the short axis. The starch grains of this variety were distinguishable from other varieties by the formation and prominence of hyla and lamellae (Bonner, 1950 and Frey-Wyssling, 1953). With the advancement of pollination date, weight and size of beans and size of starch grains became closely associated.

Concentrated Fordhook: This large seeded variety with white cotyledons was the selection from the common Fordhook variety grown in the Eastern States of America. Table 1 shows that the size of starch grains was directly related to weight and size of beans. The size of starch grains was nearly the same as those of Utah 16, Clark's Bush, and Wasatch Bush. Oblong starch grains were common (Salunkhe and Pollard, 1955a). Slightly hylumed starch grains were greater in number than highly hylumed starch grains.

Phaseolus polystachyus L.: This species occurs wild in eastern United States from Texas and Florida north to Minnesota and Main (Small, 1933). It has a purple or whitish corolla and scimitar shaped pods, about 4 to 8 centimeters long. The stem is of climbing habit. *P. lunatus* is grown widely in the United States. It has a white corolla and broad scimitar shaped pods. *P. polystachyus* has hypogeal germination, whereas *P. lunatus* has epigeal germination (Lorz, 1952).

Table 2 shows that the relationship of size and other starch grains characteristics were similar to that of previously-mentioned varieties of *P. lunatus.* The weight and size of beans were less than those of *P. lunatus.* In general, unlamellated starch grains were more frequent in this species than in *P. lunatus.* Furthermore, the percentage of unlamellated starch grains was higher in the small beans as compared to medium and large beans.

F_1 *of P. lunatus* var. *Fordhook* $\times$ *P. polystachyus*: The main purpose of this cross was to transfer the character of hypogeal germination from *P. polystachyus* to *P. lunatus.* The authors feel that there may be some possibilities for the selection of (1) a perennial cold-hardy root stock; (2) superior vegetative vigor; (3) resistance to diseases and pests and (4) higher amylose content. Furthermore, beans of this variety might be easily digestible and/or require less cooking time due to their high amylose content. Lorz (1953) attributes the very low seed production of the F_1 plant to the low ($\pm$ 1 per cent to 10 per cent) percentage of apparently good pollen produced in combination with a presumably low percentage of viable female gametes.

Table 2 shows that the weight and size of beans and size of starch grains in the F_1 were similar to *P. lunatus* and much higher than in the *P. polystachyus* parent. Other starch grain characteristics (shape, hylum, and lamella) also differed from that of both the parent species. Circular shaped, highly hylumed, and lamellated starch grains were common.

TABLE 2

The effect of size and weight of beans on the size of starch grains in species P. polystachyus *and* F_1 *cross* (P. lunatus *var. Fordhook* × P. polystachyus)

Species	Size of beans	Weight of beans (gram.)	Size of starch grains (microns)
P. polystachyus	Large	0·40	21·64
	Medium	0·25	18·48
	Small	0·11	14·45
		$*\hat{y}=11\cdot99+24\cdot76x$	
P. lunatus × *P. polystachyus*		0·84	31·58

* Regression of starch grain size (μ) on seed wt. (gm.).

GERMINATION STUDIES

P. lunatus and *P. polystachyus* had epigeal and hypogeal germination respectively (Fig. 1), whereas the F_1 plants were intermediate between the parents (Fig. 2). Efforts are being made to have the hypogeal character transferred completely to the *lunatus* parent by back crossing. Due to the high gametic sterility in the F_1, it is difficult to get successful back-crosses with the *lunatus* parent.

AMYLOSE CONTENT

Amylose content in the two species, regardless of the varieties studied, was approximately 24 per cent. Utah 16, and Clark's Bush possessed the same amount of amylose regardless of the size groups of beans. Wasatch Bush, Concentrated Fordhook, and *P. polystachyus* showed an apparent trend towards a lower percentage of amylose in large beans. As has been pointed out previously, large beans had large-sized starch grains and nearly regular shape, whereas small beans had small-sized starch grains which were slightly irregular in shape. These results agree with the results found in corn, that high-amylose corn hybrids possessed higher percentage of smaller, irregular starch grains (Senti, 1958).

The cross between *P. lunatus* var. Fordhook and *P. polystachyus* showed 15·06 per cent amylose in the F_1 beans, which was about one-half that of both the parent species. The bean from which amylose was determined was large, weighing 0·84 gm., with average starch grains size of 31·48 microns, and had 63 per cent circular starch grains. Keeping the above-mentioned statement in view, it was concluded that one reason of low per cent of amylose might be the large circular starch grains (Senti, 1958); another, the genetic nature. At this stage, it is difficult to say anything about the exact genetic behaviour.

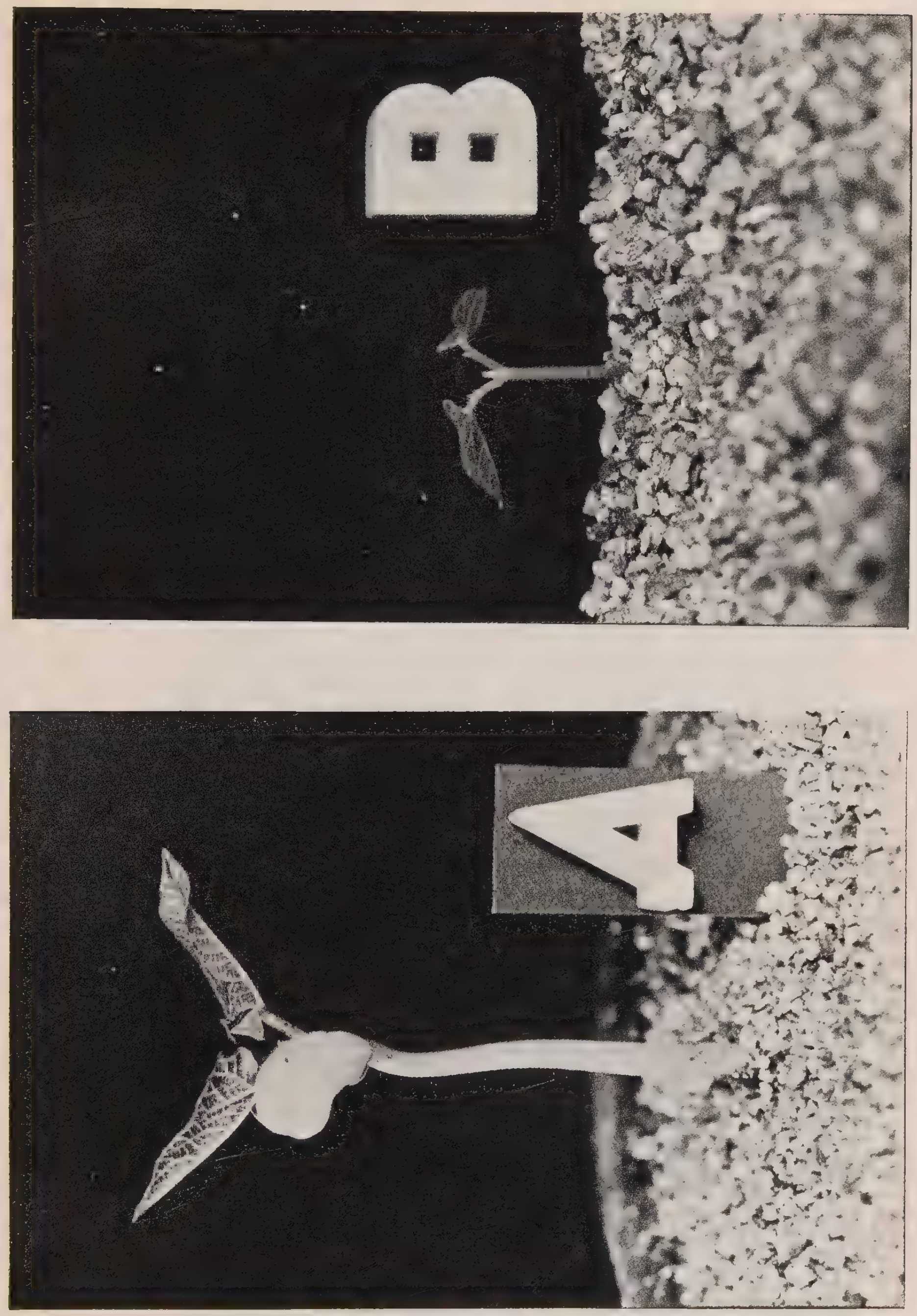

Fig. 1. Germination behaviour of *Phaseolus lunatus* var. Fordhook (A) and *Phaseolus polystachyus* (B).

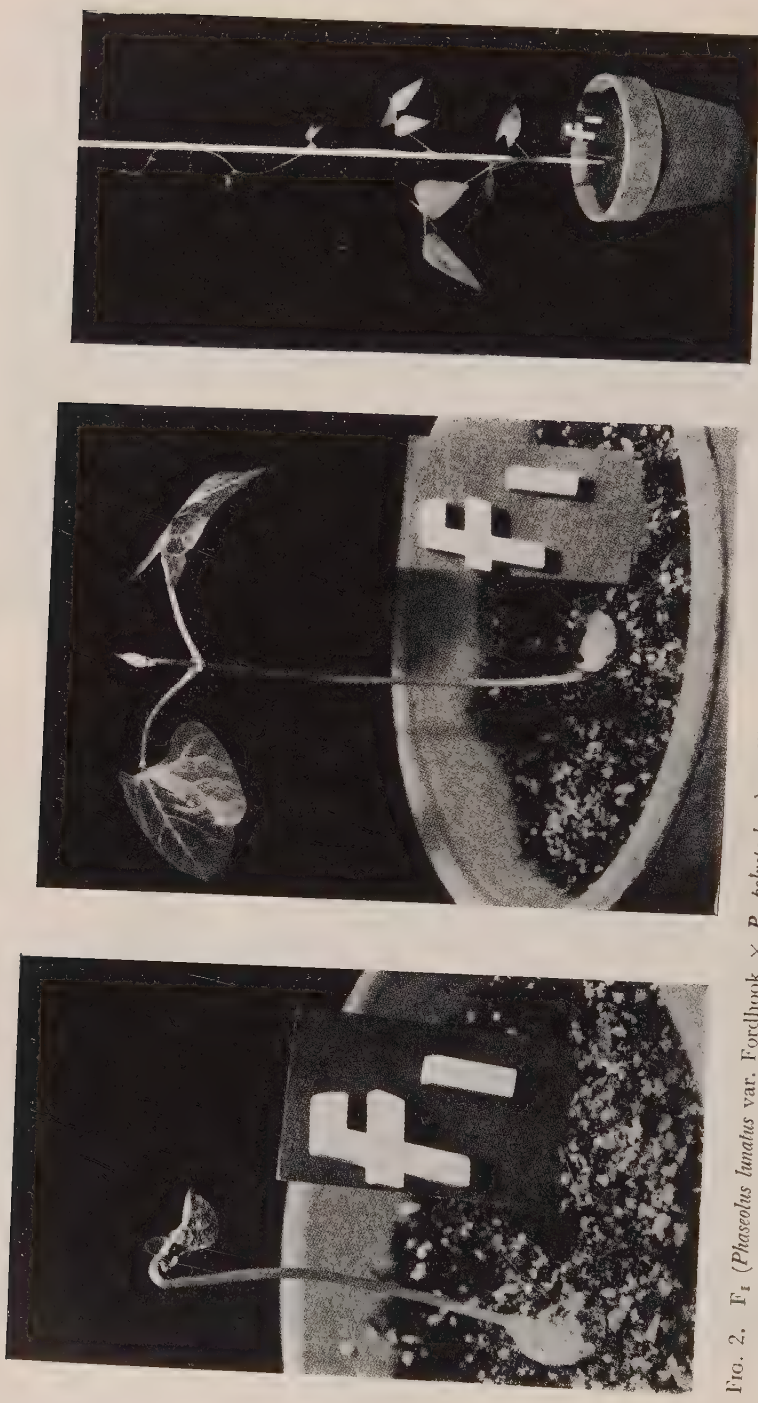

FIG. 2. F_1 (*Phaseolus lunatus* var. Fordhook × *P. polystachyus*) seedlings showing three subsequent stages of growth and cotyledon location.

Summary and Conclusions

Some characteristics of the starch grain were studied in 5 varieties of *Phaseolus lunatus*, in *P. polystachus* and in the F_1 of the cross between the two species, grown at Farmington, Utah.

The results obtained indicated that species and varieties of *P. lunatus* differed in starch grain size and other characteristics. Burpee Bush and Concentrated Fordhook were large seeded varieties, possessing large starch grains. Varieties Utah 16, Clark's Bush, and Wasatch Bush were small-seeded and possessed smaller starch grains as compared to the large-seeded varieties. *P. polystachyus* had small seeds containing small starch grains which were more or less like those in the small-seeded varieties of *P. lunatus*. The F_1 seeds (*P. lunatus* var. Fordhook × *P. polystachyus*) and starch grain size were similar to that of the large seeded varieties of *P. lunatus*.

As the pollination time advanced the size of the starch grains decreased. Size and weight of beans had linear relationship with the size of starch grains.

Amylose content was more or less equal (24·44 per cent) in both species but, decreased to 15·06 per cent in the F_1.

The F_1 was intermediate between the parents in the type of germination.

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TECHNIQUES FOR IDENTIFYING VARIETIES OF *BETA VULGARIS* IN THE LABORATORY

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For the seed trade and in seed testing work it is of great importance to distinguish the seeds of sugar beetsfrom those of sugar fodder beets. Through wrong identification or mixed seeds, the sugar industry has often suffered serious losses. When the mixed crop is already growing in the field, it is too late to do anything to reduce the loss. It would therefore be of great value, if the identification of types and varieties could be undertaken in the laboratory on seeds, clusters or early sprouts in the usual routine way.

According to Pieper (1919) the sugar beets can be distinguished from fodder beets in about 7 days on the basis of hypocotyl colour of the germinating plants. However Pieper (1921) as well as Griessmann (1931) and especially Eggebrecht (1941) have reported that this characteristic of hypocotyl colour fails in the distinction between sugar and sugar-fodder beets, as both groups produce pink as well as greenish-white sprouts.

Munerati and Milan in 1927 (quoted from Ludwig, 1954) observed, under optimum temperature and light conditions in a glass house, that the young sugar beet plants had at least one growth ring more than the sugar-fodder beets. This criterion, however, can only be applied to plants grown under uniform conditions. For practical purposes, therefore, it is likely to be of very limited importance.

According to Ludwig (1954), it should be possible to differentiate between sugar beets, sugar-fodder beets and fodder beets on the basis of the differences in the sugar content of the seedlings. This method, has, however, not been confirmed by any other worker so far.

The present investigation deals with this problem of differentiating the varieties of *Beta vulgaris*. Experiments were undertaken to check the possibilities of finding out a method for the identification of the seeds of sugar beets and sugar-fodder beets in the laboratory, in a short time.

Material

As against the rich selection of sugar beet varieties, the number of sugar fodder beets was limited to three till recently. Out of these, the variety " Veni Vidi Vici " possesses totally white sprouts and can hence be easily distinguished from the other two varieties of sugar fodder beets. The present investigations were therefore conducted with the sugar fodder beet varieties " Ovana " and " Rheinische Lanker ". In addition, Mangold (*Beta vulgaris* var. *cicla*) was also included in the investigations, as it cannot be differentiated from the other *Beta* varieties on the basis of hypocotyl colour.

Results

1. *Treatment of seeds with Streptomycin sulphate solutions:*

Trials were set up with different concentrations of Streptomycin sulphate in order to see if a differentiation of the types of *Beta vulgaris* could be made through the action of this chemical in the early stages of seed germination.

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The samples were sorted out into different sizes of clusters with the help of a grading machine, using the standard methods of seed testing (after Eggebrecht 1949) and from each type an average sample of 4×100 clusters was taken. The treatment was given for 16 hours in petri-dishes containing 20 cc. of Streptomycin sulphate solutions of different concentrations. Afterwards, the clusters were washed with tap water and spread for germination on filter paper, wetted with 15 c.c. of tap water, in Polystyrol Germination Trays. The germination test lasted for 14 days and the germinated seeds were counted after every 3 days in the first week and after every 4 days later on. The results are summarized in Fig. 1.

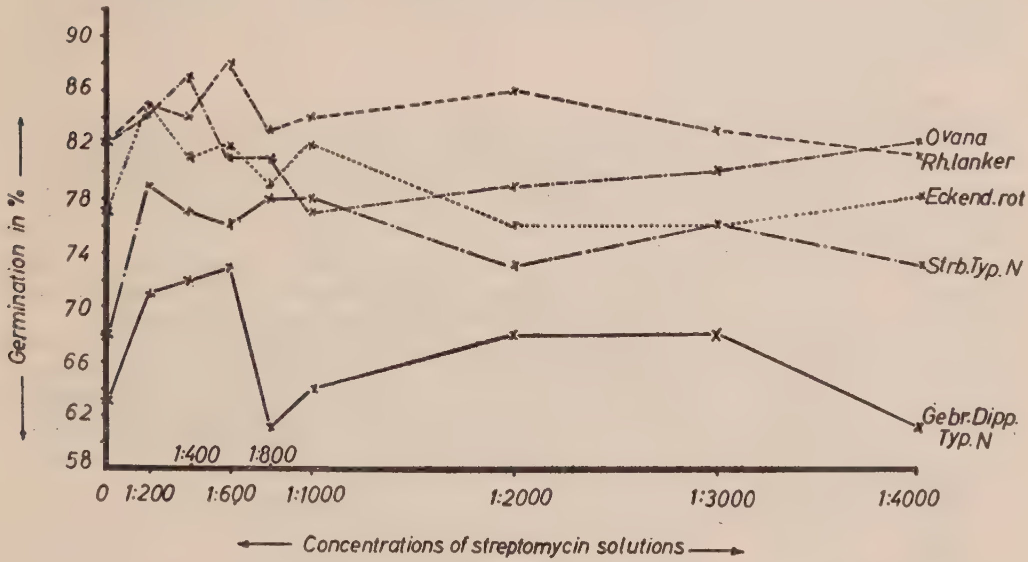

FIG. 1. Effect of various concentrations of Streptomycin sulphate on different types of *Beta vulgaris*.

It will be observed from the curves that Streptomycin sulphate has only a weak action on the germination of *Beta* seeds. Although there is a rise in germination percentage in all the tested samples treated with Streptomycin at a concentration of 1:200, only the values for sugar beets, "Gebr. Dippe Type N", "Strubes Type N", and the fodder beet, "Eckendorfer rot", differ from the untreated samples by more than the 4-5 per cent margin, generally used in seed testing. The sugar fodder beets, "Ovana" and "Rheinische Lanker", show an increase in germination only with solutions of 1:400 and 1:600. The remaining concentrations have had practically no effect on germination. The differences in germination values in different samples tested are, however, so small, that it is not possible to draw any reliable conclusions for the identification of varieties of *Beta vulgaris* by this method.

2. *Soaking in water:*

In order to find out if the seeds of sugar and sugar fodder beets react physiologically differently to soaking in water, a separate set of experiments was started. The seeds were soaked in tap water for two, three, seven and eight days and then allowed to germinate on filter paper in the same way as in the previous experiment, and in quartz sand at 20°C. The results are presented in Figs. 2 and 3.

As against the controls, all the treated samples showed a decrease in the germination percentage after soaking for two days. Then there is a slight rise in germination percentage in all the samples except in the fodder beet " Eckendorfer gelb ", followed again by a uniform fall upto the 7th day of the treatment. The rise in the germination percentage after 8 days of soaking is, however, very unusual. The experiment was stopped at this stage, as there were no marked physiological differences useful in the identification of different types.

In any case, it has become clear that *Beta* seeds can remain in water for a long time without losing their viability completely. The results of this trial confirm the observation made in the case of treatment of different species of seeds with absolute alcohol (Kamra, 1959) that the viability of seeds does not fall continuously but exhibits fluctuations.

3. *Treatment with $KClO_3$* :

As a result of his investigations, Yamasaki (1957) reported that a differentiation of varieties of wheat, rice and barley can be made on the basis of the damage suffered by the seeds and seedlings after treatment with $KClO_3$ solution for a definite time. Trials were, therefore, made to see whether the different varieties of *Beta vulgaris* could also be distinguished in a similar way.

(a) *Treatment of seeds.*—The *Beta* clusters were soaked in a 3 per cent solution of $KClO_3$ for 48 hours at 25°C. Thereafter, they were washed with running water and put on filter papers in petri-dishes for germination at room temperature.

After 15 days it was observed that the roots of the treated plants had died and turned dark, whereas the stems were still alive and green. The control samples showed a similar behaviour where the roots died because of an attack by *Fusarium*. Chemical treatment of seeds against the attack of pathogens did not seem advisable as

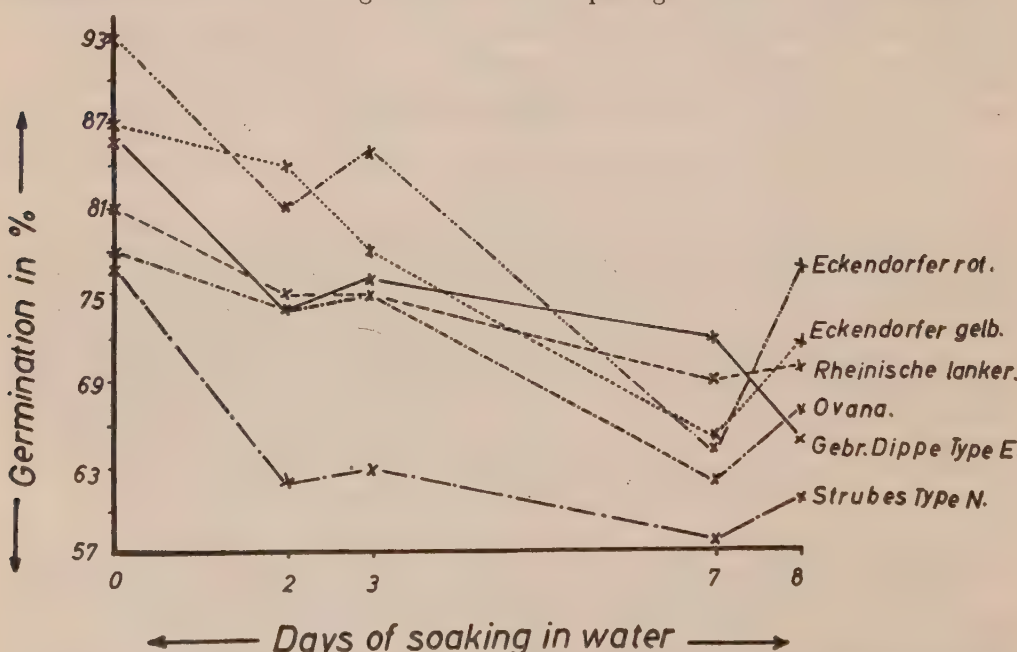

FIG. 2. Effect of the period of soaking in water on the germination capacity of *Beta vulgaris* seeds (Filter Paper Series).

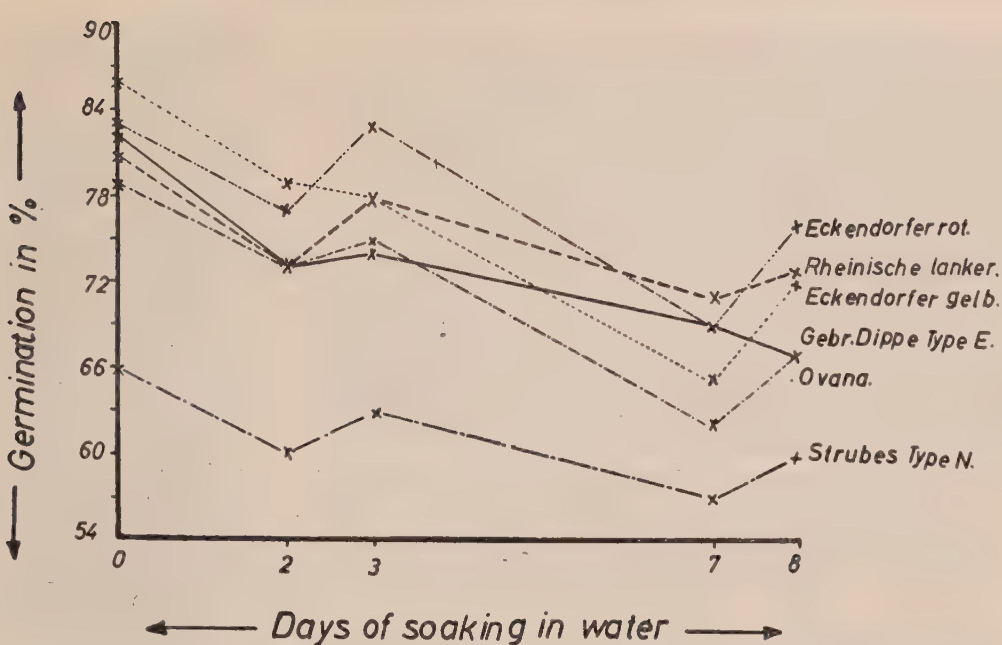

FIG. 3. Effect of the period of soaking in water on the germination capacity of *Beta vulgaris* seeds (Sand Series).

through that the experiment would have been disturbed. This method did not give any useful clues towards the solution of the problem.

(b) *Treatment of seedlings.*—The seeds were germinated on filter paper at normal room temperature and then treated with 1 per cent $KClO_3$ solution for 24 hours by dipping the roots completely in the solution. The control samples were treated with water for the same time.

As in the earlier experiment, the roots of the treated samples died here too but the stem parts remained normal. The control showed a much higher percentage of healthy seedlings than the treated sample. Although the seedlings of the sugar beets had been damaged a little more severely than those of the sugar-fodder beets, the differences were not very marked.

It must, therefore, be concluded, that treatment with $KClO_3$ cannot also be employed for the differentiation of *Beta* types.

4. *Treatment with absolute alcohol:*

As the damage to seeds through alcohol depends upon their water content, they were dried at 35°C for 4 days in order to reduce their moisture value to 5-6 per cent. The seeds were treated with absolute alcohol in properly cleaned, dried, stoppered bottles for two hours at room temperature and afterwards carefully dried for half an hour at room temperature to remove the extra alcohol. They were germinated on filter paper in Polystyrol germination trays. The differences between different varieties in respect of loss of germination were, however, so small that a method could not be correctly based upon them.

5. *Investigation of root fluorescence:*

For this purpose seeds were germinated in special glass tubes (Fig. 4). These

tubes are 23 cm. long and have a diameter of 0·9 cm with a constriction 2·5 cm from the upper edge, which serves as a resting place for the germinating seeds. (For further details see Kamra, 1958a). The fluorescence observations were made on 14-day old seedlings. This period turned out to be the best as compared with the earlier or later dates. It was observed that the roots of sugar beets ("Gebrueder Dippe Type E", "Strubes Type N") showed a light blue-white, those of the sugar-fodder beets ("Ovana", "Rheinische Lanker") light blue-green and those of fodder beets ("Eckendorfer rot" and "Eckendorfer gelb") light green, fluorescence. These fluorescence colours could be seen a little clearer when an orange filter (Schott & Gen. G 111) was used for observation. The side roots of sugar beets showed a distinct yellow fluorescence; this fluorescence was of much less intensity in case of sugar-fodder beets.

It is evident from the above results, that small differences in fluorescence colours are visible. They can give useful clues for the distinction between sugar, sugar-fodder and fodder beets.

6. *Photometric measurements of the liquid in which the roots of the seedlings had been growing:*

Tap water, in the tubes described above, was analysed after the roots had grown in it for 4 or 5 weeks with an Eppendorf-Photometer using fluorometric attachment and special filters. The results showed that although there were notable differences between the liquid in which roots had been growing and the controls (water lying in similar tubes without plants), the inter-varietal differences were so small that an identification of types of *Beta vulgaris* could not be achieved through this method.

7. *Refractometric measurement of the sugar content of the seedlings:*

As described by Ludwig (1954) the Beta clusters, treated with the disinfectant Germisan for 1 hour, were put on a 4 cm., thick layer of sterile quartz-sand (particle size 1·0 to 1·5 mm.) in glass dishes of 12 cm. diameter and covered with about 1 cm. thick layer of sand. The sand was saturated with a mixture of Knop's and Hoagland's A-Z culture solutions. These dishes, covered with glass plates, were kept in a glass house at 25°C and were brought to their original weight by addition of distilled water every 2-3 days.

After 14 days it was observed that out of 25 *Beta* clusters put into each dish, only very few had germinated (Fig. 5). A better germination was perhaps not to be expected, as due to thick, moist sand around the seeds, there was lack of oxygen. Under normal conditions, the same sample showed a much higher percentage of germination. It, therefore, seemed necessary to change the method of Ludwig (1954) in this respect.

In subsequent trials, the thickness of the sand layer above the seeds was reduced from 1 cm. to 1-2 mm., and the sand was kept moderately moist instead of saturating it with culture solutions. In addition, 50 clusters were sown in each glass dish for germination. Through these changes a better germination and a higher number of seedlings was obtained as is seen in Fig. 6.

The measurement of sugar content was made on 29 days old seedlings with an Abbe's Refractometer (Carl Zeiss). This refractometer has the advantage over the hand refractometer (used by Ludwig) in that the temperature during the period of measurement can be kept constant. The plants were kept in darkness for one day before investigation, as prescribed by Ludwig and were carefully taken out of the sand and washed with water to remove the remaining sand particles. The juice was pressed out of the plants only after they had been properly cleaned and the additional water on them removed with the help of filter paper. The results are summarized as follow:

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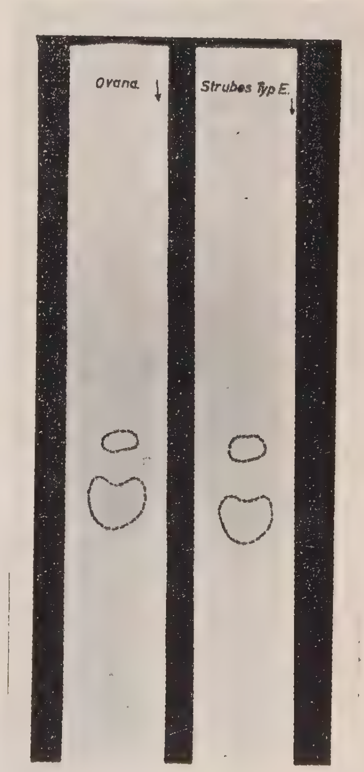

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Fig. 4. Special type of tubes for growing individual seedlings.
Fig. 5. Growth of seedlings in quartz-sand according to the method of Ludwig (1954) (explanation in the text).
Fig. 6. Improvement in growth as a result of certain alterations in the above method.
Fig. 7. Paper-chromatograms of a sugar beet and a sugar-fodder beet variety.

TABLE 1

Sugar content in 29 *days old seedlings of* Beta vulgaris.
(Temperature during the period of measurement 20°C constant)

Type		Sugar content Average of 100 measurements each
I. Sugar beets:		
Gebrueder Dipp Type Z	..	2·312%
Strubes Type N ..	..	2·303%
II. Sugar-fodder beets:		
Ovana	..	2·324%
Rheinische Lanker ..	..	2·361%

It is clear from the results, that no marked difference in the sugar content of the seedlings of different types are observable. A distinction between sugar and sugar-fodder beets cannot, therefore, be made in this way.

8. *Paperchromatographic investigations:*

The observation that the seedlings of sugar and sugar-fodder beets do not show any marked differences in sugar content was confirmed by the paper-chromatographic investigations of the same. The sugar chromatograms of both types (Fig. 7) hardly showed any differences.

9. *Studies on the ratio of the hypocotyl colours in the seedlings:*

A useful clue for the identification of *Beta* types was obtained through the investigations of the hypocotyl colour proportions in the seedlings. The seeds were sown on 4 cm. thick, moderately wet, quartz sand in glass dishes (diameter 12 cm.) and covered with a layer of sand, 1-2 mm. thick. These dishes, covered with glass plates in order to maintain a high degree of moisture in the first week, were put in a glass house at 20°C and 70 per cent relative humidity in diffused daylight. The glass plates were removed as soon as the cotyledons came out. The seedlings were watered every two days and the hypocotyl colour was determined after 14 days.

The results (Fig. 8) show that the sugar-fodder beet variety " Ovana " deviates from all the other investigated sugar and sugar-fodder beets in the very high percentage (97·8 per cent) of pink coloured seedlings produced and hence can be distinguished from them. The sugar-fodder beet type " Rheinische Lanker " shows, on the other hand, a very small percentage (30·2 per cent) of pink coloured plants. Because of the fact that the percentage of pink coloured seedlings in the varieties of sugar beets tested, except for the variety " Dieckmanns Type N ", lie between the two above mentioned extreme values of the sugar-fodder beets " Ovana " and " Rheinische Lanker ", it is possible to differentiate between them and these varieties of sugar-fodder beets. The proportion of pink coloured seedlings in most sugar beets does not reach that of the sugar-fodder beet " Ovana ", but clearly exceeds that in the variety " Rheinische Lanker ". These observations indicate the importance of hypocotyl colour in differentiating sugar beets from sugar-fodder beets.

FIG. 8. The proportions of pink and green-white hypocotyls in the seedlings of the different varieties of *Beta vulgaris* investigated.

Mangold:

Mangold is a variety of *Beta vulgaris*. At present there is no method of distinguishing it from the sugar and sugar-fodder beets. The German Official Seed Types List contains the following four varieties of Mangold: (1) Lukullus (2) Krauser Silber (3) Gruener Schnitt (4) Glatter Silber. As a distinction in these cases on the basis of hypocotyl colour cannot be achieved, it was necessary to look for other characteristics for an identification in a short time.

For this purpose, 100 clusters of each of the above types were sown in uniform soil separately in pots (diameter 14 cm.) and kept in a glass house at 25°C. In as short a time as 7-10 days, it was observed that the distinction of these types could be made on the basis of the intensity of green colour of the cotyledons. The type " Lukullus " showed a distinct yellow-green colour of the cotyledons as against the dark-green of the other mangold types and the sugar, sugar-fodder and fodder beet varieties.

The same experiment was repeated under artificial light conditions at 18-20°C. The colour differences mentioned above were visible here too.

On the basis of these observations, it is not only possible to differentiate Mangold (*Beta vulgaris* L. var. *cicla*) in seedling stage from sugar-fodder and fodder beets (*Beta* (*vulgaris* L.) and sugar beets (*Beta vulgaris* L. var. *altissima* Roessig) in a short time, but also the variety itself can be identified with certainty without any difficulty both under natural as well as under artificial light conditions. This finding is one of the few cases in which a variety can be identified with certainty on the basis of a colour characteristic and is therefore of great importance for seed testing work and for the practice.

DISCUSSION

The above mentioned investigations on *Beta vulgaris*, through treatment with Streptomycin sulphate, soaking in water, influence of $KClO_3$ solutions, action of absolute alcohol, photometric measurements, refractometric and paper-chromatographic determinations of sugar content etc., showed that a distinction between the

seeds of sugar and sugar-fodder beets cannot be made by using these techniques. The results of Ludwig (1954) on the sugar content of the seedlings could not be confirmed. He has given the refractometric values for the sugar content of 29 days old seedlings of the sugar beet "Type N" and the sugar-fodder beet variety "Ovana" as 4·34 per cent and 2·69 per cent respectively and remarks that the values for sugar content of the seedlings of sugar-fodder and fodder beets lie about 30 per cent below those of seedlings of sugar beets. However, the refractometric measurements conducted in the course of the present investigations on equally old plants, nourished in the same way, did not show any observable differences in the sugar content of the seedlings. This observation was also confirmed by the paper-chromatographic method.

About the use of the percentage proportions of hypocotyl colours of the seedlings as a character for the identification of sugar and sugar-fodder beets, Eggebrecht (1941) has expressed the following view: "This characteristic fails in the case of white beets, Lanker and Ovana, because they develop pink and white sprouts side by side, as in the case of sugar beets". As against this view, the results of the present experiments show that the sugar-fodder beet varieties "Ovana" and "Rheinische Lanker" can be distinguished with some certainty from the sugar beets tested on account of the differences in the percentage of pink coloured seedlings in them. For further confirmation of the types, the fluorescence of roots can be studied.

In conclusion it may be said, that certain difficulties still exist in the identification of seeds of sugar and sugar-fodder beets in laboratory. However, it is possible to obtain useful indications for this purpose by combining a number of different characters. On the basis of the present investigations, the precentage of seedlings with pink hypocotyles and the different types of root fluorescence, would appear to be useful characters.

Summary

The present paper deals with the problem of differentiation between seeds of sugar-fodder and sugar beets in laboratory. The investigations also included Mangold (*Beta vulgaris* var. *cicla*).

Treatments of seeds with Streptomycin sulphate solutions, soaking in water, action of absolute alcohol, photometric measurement of the liquid in which the roots of the seedlings had been growing, refractometric and paper-chromatographic determinations of the sugar content of the seedlings, led to negative results.

A valuable clue for the differentiation of types was obtained through the distribution of hypocotyl colours in seedlings. The root fluorescence also showed small differences among the *Beta* types.

For the identification of Mangold seeds from those of other *Beta* varieties, a difference in the cotyledon colour proved useful.

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MORPHOLOGICAL AND CYTOLOGICAL EFFECTS OF RADIOACTIVE PHOSPHORUS AND RADIOACTIVE SULPHUR IN SOME CEREALS AND LEGUMES

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A study of the cytological and immediate morphological effects of treatment with radioactive phosphorus (P^{32}) and radioactive sulphur (S^{35}) was undertaken to evaluate the cytological effects of low doses of beta radiation and the claims of some workers that low radiation levels stimulate crop growth. In addition, it was hoped that such a study will provide useful data concerning the determination of dosage thresholds to be adopted in large scale experiments for inducing mutations in these plants. The importance of mutation breeding in solving specific problems facing the plant breeder is well known; Gustafsson and Tedin (1954) and Gustafsson (1957) have summarised the role of radiation in plant breeding.

MATERIAL AND METHODS

A variety each of Bread wheat (*Triticum aestivum*) and paddy (*Oryza sativa*) among cereals and of Broad Bean (*Vicia faba*), Guar (*Cyamopsis tetragonoloba*), Berseem (*Trifolium alexandrinum*) Red clover (*Trifolium pratense*) and Cowpea (*Vigna sinensis*) among legumes were used in this study. Radioactive phosphorus and radioactive sulphur were imported from the Radiochemical Works, Amersham, England. Both seed treatment, i.e., allowing the seeds to germinate in a known quantity of radioactive solution at the dosage rate required and transplanting after 48 hours of treatment, and soil treatment, i.e., mixing the required quantity of radioactive isotope in soil at the rate of 8 lbs. of soil per pot were adopted. Cytological studies were made in Feulgen or Orcein squash preparations following acetic-alcohol fixation.

OBSERVATIONS

Cereals:

To study the cytological effects of the same dosage of P^{32} on paddy and wheat, dry seeds were treated with 5 μC and 10 μC of P^{32} and S^{35} per seed and the root tips were fixed on the day of emergence. Chromosome and a few chromatid breaks at metaphase and bridges, both sticky and dicentric, at anaphase, were recorded. The frequency of aberrant cells in the root tips is given in table 1.

In another experiment paddy seeds were treated with 1, 2, 3, 5, and 10 μC of P^{32} and it was found that those plants treated with the highest dosage, were more vigorous and taller than those treated with lower dosages. However, they were not better than the controls. In an experiment, where the treatment was given through the soil at the doses 2·72 μC, 5·45 μC and 10·9 μC, the plants treated with 10·9 μC flowered 8 days earlier than the control.

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TABLE 1

Frequency of aberrant cells in root tips obtained from paddy and wheat seeds treated with radioactive isotopes

Material	Dose per seed	Duration of treatment	Period of recovery	Percentage of aberrant cells	
				P^{32}	S^{35}
Wheat	5μC	48 hours	24 hours	38·0	35·0
	5μC	48 hours	72 hours	37·0	21·6
	10μC	48 hours	24 hours	68·5	50·8
	10μC	48 hours	72 hours	37·0	33·7
Paddy	5μC	48 hours	24 hours	0·0	5·0
	5μC	48 hours	72 hours	0·0	0·0
	10μC	48 hours	24 hours	5·8	0·0
	10μC	48 hours	72 hours	0·0	0·0

Pollen sterility was estimated in the control as well as treated paddy plants grown in the Radiotracer Laboratory of this Institute. Two different soils, i.e., soil from Jasalpur and soil from Karjat had been used in each experiment. The data are presented in table 2.

TABLE 2

Pollen sterility in paddy plants exposed to different doses of β-radiation

Treatment	Percentage of sterile pollen in	
	Plants grown in soil from Jasalpur	Plants grown in soil from Karjat
Control	6·9	11·76
2.72μC	17·1	28·80
5.45μC	15·84	18·21
10.90μC	33·6	39·2

The pollen sterility data were statistically analysed and it was found that the difference between the treatment and controls was significant.

Pollen size was also found to vary much in the different treatments and smaller size of pollen grains was found to be associated with sterility. Three classes could be recognized according to size i.e., small, medium and large, corresponding to 28·8, 32·4 and more than 36·0 microns respectively. The data are presented in table 3.

TABLE 3

Pollen size in paddy plants exposed to different doses of β-radiation

Soil	Treatment	Percentage of grains		
		Small $28 \cdot 8\mu$-$32 \cdot 4\mu$	Medium $32 \cdot 4$-$36 \cdot 0\mu$	Large 36μ and above
Jasalpur ..	Control	19·3	61·4	19·6
	$2 \cdot 72\mu C$	21.7	66·6	11·6
	$5 \cdot 45\mu C$	29·4	46·0	29·0
	$10 \cdot 90\mu C$	39·2	51·1	9·6
Karjat	Control	20·8	35·2	43·3
	$2 \cdot 72\mu C$	29·5	49·0	20·9
	$5 \cdot 45\mu C$	22·7	54·5	22·8
	$10 \cdot 90\mu C$	50·8	44·0	5·2

The frequency of occurrence of small-sized pollen grains is maximum in the 10·9 μC dose and least in the control.

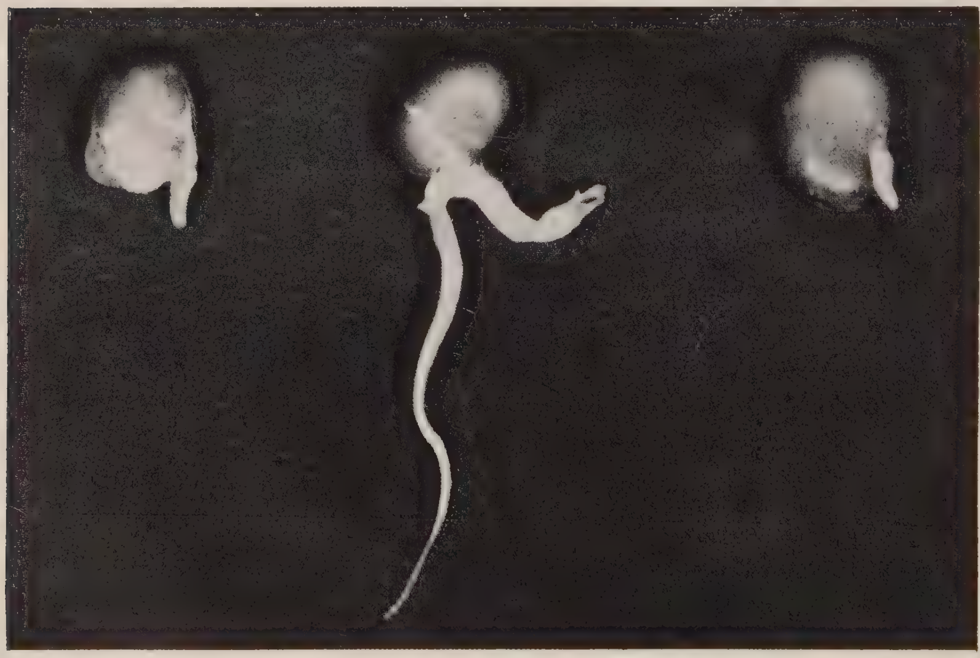

FIG. 1. Showing inhibition of root growth in *Vicia* (Control in the centre).

Legumes

Experiments with P^{32}:

In *guar*, there was stimulation of growth in plants grown from seeds treated with P^{32} (Fig. 2). The average height of plants after various intervals is given in table 4.

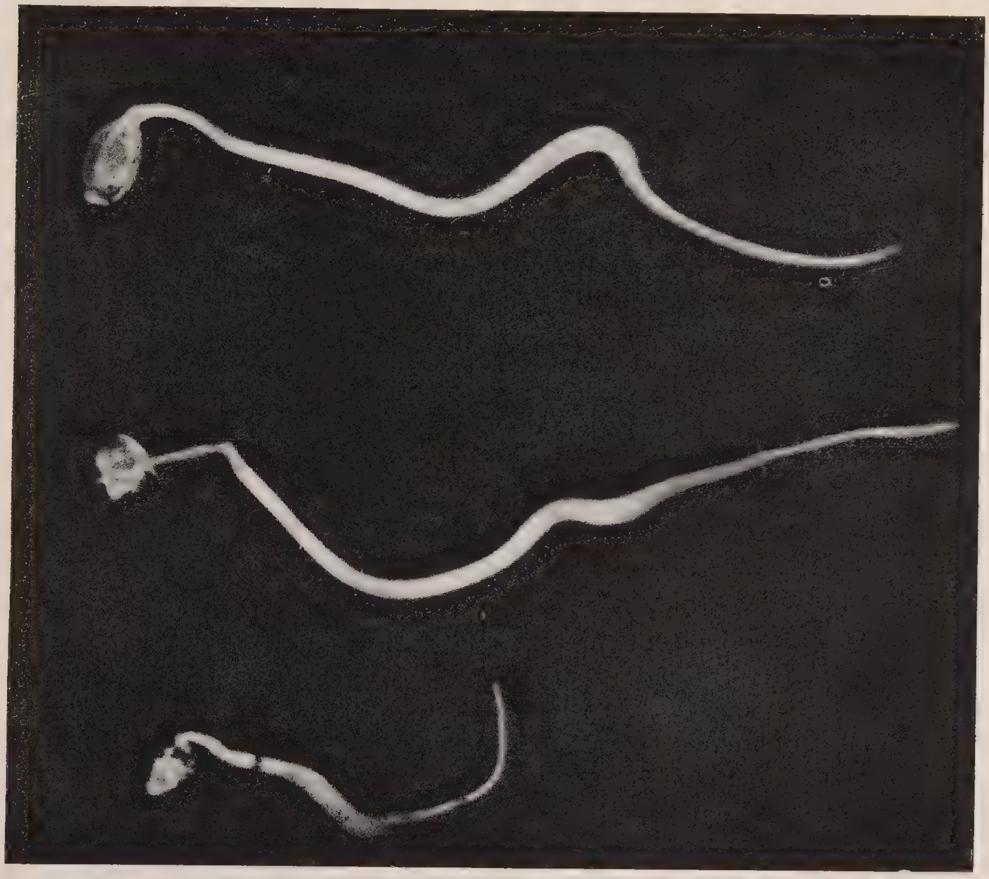

Fig. 2. Showing stimulation of root growth in *Guar* (2nd and 3rd from bottom treated).

Table 4

Height of control and treated guar *plants at different stages of growth*

	Average height in cm. after			
	5 days	11 days	20 days	At flowering
Control	0·8	2·0	4·0	43·0
Treated ..	1·2	2·3	5·1	55·1

In both cowpea and *guar*, the leaves of treated seedlings showed many irregularities in shape and texture, like suppression of one cotyledon and fusion of two leaflets. Further, the leaves presented a highly puckered and crinkled appearance with a tough and leathery texture. About 4·6 per cent of control and 23·9 per cent of the treated plants showed puckered leaves.

In all the legumes studied (except *Vicia faba*) it was not possible to analyse critically the root tip cells for chromosome breaks at metaphase due to the extremely small size of the chromosomes. However, none of the clear cells showed any abnormalities at metaphase. Abnormalities at anaphase were analysed and the data are presented in table 5. Stickiness of chromosomes was much more pronounced in the legumes as compared to the cereals. Dicentric bridges as well as sticky bridges were observed in some of the cells of treated leguminous seedlings.

TABLE 5

Percentage of aberrant cells at anaphase in root tip cells of some legumes treated with radioactive isotope, P^{32}

Material	P^{32} dose	Period of recovery (in hours)	Total No. of Cells analysed	No. of aberrant cells	% of aberrant cells
Guar ..	5μC	24	24	2	8·3
	5μC	72	32	0	0·0
	10μC	24	32	2	5·7
	10μC	72	22	0	0·0
Berseem (2n) ..	5μC	24	12	0	0·0
	5μC	72	13	0	0·0
	10μC	24	6	0	0·0
	10μC	72	75	1	1·3
Berseem (4n) ..	5μC	24	30	1	3·3
	5μC	72	16	0	0·0
	10μC	24	24	4	16·6
	16μC	72	13	2	15·3
Red clover ..	5μC	24	19	0	0·0
	5μC	72	17	0	0·0
	10μC	24	10	0	0·0
	10μC	72	13	0	0·0

Meiosis was studied only in *Guar*. The only observable effect was stickiness of chromosomes seen in some cells. Meiosis was otherwise normal. The analysis of tetrads also did not show any marked abnormalities. Pollen fertility was found to be normal with only about 3·6 per cent of sterile pollen as against 2·5 per cent in controls.

Experiments with S^{35}:

The seeds of the legumes under study were treated with 5 μC and 10 μC per seed of S^{35} and the immediate growth response was studied. The average length of the primary roots was measured and the data are presented in table 6.

TABLE 6

Growth response of some legumes treated with S^{35}

Material	Average length of primary roots in Cm.		
	Control	5μC/seed	10μC/seed
Berseem (2n)	1·9	1·9	2·2
Red Clover	0·9	2·0	2·4
Guar	1·0	2·9	1·8
Vicia faba	2·5	0·8	1·0

It will be noted that, except in the case of *Vicia*, there was a stimulation of root growth. Root tip mitoses were studied in these treated plants. Due to the small size of the chromosomes it was rather difficult to analyse the metaphase plates. However, anaphase abnormalities like dicentric bridges, sticky bridges and chromosome and chromatid breaks were recorded and the data are presented in table 7.

TABLE 7

Root tip mitoses in legumes treated with S^{35}

Material	S^{35} Dosage (per seed)	Period of recovery (in hours)	Total No. of cells	No. of aberrant cells	Percentage of aberrant cells
Vicia faba ..	5μC	24	30	12	40·0
	5μC	72		No division	
	10μC	24	41	20	48·7
	10μC	72		No division	
Guar ..	5μC	24	28	0	0·0
	5μC	72	34	0	0·0
	10μC	24	33	1	3·0
	10μC	72	23	1	4·3
Berseem (2n) ..	5μC	24	28	1	3·5
	5μC	72	34	0	0·0
	10μC	24	30	2	6·3
	10μC	72	46	0	0·0
Red Clover ..	5μC	24	14	0	0·0
	5μC	72	16	0	0·0
	10μC	24	32	1	3·1
	10μC	72	26	0	0·0

Discussion

From this preliminary study, some points of interest emerge. Large scale experiments are in progress with the use of radioactive isotopes as tracers, and it is interesting to study whether the dosages of P^{32} and S^{35} used for tracer experiments induce any marked cytological and genetic changes in the treated material. Tracer dosages vary from $2{\cdot}7\mu C$ to $10{\cdot}9\ \mu C$. It is seen that these dosages induce chromosomal aberrations as well as certain physiological changes which either suppress or stimulate growth. So it is worth while to study in detail the various effects of such low, continuous radiation in biological systems and how far these effects may affect the interpretation of results obtained by tracer studies, especially those pertaining to physiological characters like uptake, distribution, and utilisation of nutrients, carbohydrate metabolism etc. It would be worth while to determine the safe limit for each crop before such tracer experiments are undertaken. Secondly, it is clear from this study that different plants respond differently to the same dosage of radioactive isotope. A differential response of crop plants to X-rays has been reported by Gustafsson (1944) and to chronic gamma radiation by Sparrow (1954). In the present experiment, it is seen that wheat which has long chromosomes are affected more than paddy which has small chromosomes. Similarly the larger chromosome length may account for the high sensitivity of *Vicia faba* in comparison to other legumes like *guar* or cowpea, the chromosomes of which are relatively smaller.

Another factor which may account for this differential response is difference in the size of embryo or seed. Fröier and Gustafsson (1944) reported that an increase in seed size and embryo size caused better germination and growth after X-irradiation. They concluded that plants with large cells are less sensitive. The seed size and embryo size of the plants studied here and their relative biological response are given in table 8. It can be seen from this table that no simple relationship between seed or embryo size and radio-sensitivity exists in the material studied.

Table 8

Relationship between seed size and response to treatment with radioactive isotopes

Material	Seed		Embryo		Cytological aberrations at	
	Length	Breadth	Length	Breadth	5 μC	10 μC
	Cms.	Cms.	Cms.	Cms.		
Wheat ..	0·6	0·3	0·35	0·15	High	Drastic
Barley (2n) ..	0·9	0·3	0·45	0·13	Negligible	Low
Barley (4n) ..	1·2	0·4	0·50	0·20	Medium	Medium
Paddy ..	0·9	0·2	0·15	0·10	Nil	Negligible
Vicia faba ..	1·3	0·8	0·55	0·28	Drastic	Drastic
Guar ..	0·5	0·4	0·13	0·05	Negligible	Negligible
Berseem (2n) ..	0·2	..	0·45	0·10	Nil	Negligible
Berseem (4n) ..	0·3	..	0·60	0·12	Nil	Low
Red clover ..	0·2	..	0·40	0·13	Nil	Nil

Another variable which may affect the sensitivity of seeds is the rate of absorption of the isotope and mode of translocation. Since absorption is controlled by various external factors like temperature, humidity etc., it will be rather difficult to

draw precise inferences concerning the cause of the observed difference in sensitivity in a given treatment with radioisotopes.

Summary

A preliminary study has been made of the effects of radiophosphorus and radiosulphur on growth and morphological characters, somatic cell division, and pollen and seed fertility in paddy and wheat amongst cereals and *Vicia*, *Guar*, *Cowpea*, Berseem and Red clover in legumes. The biological effects of tracer dosages of radioactive isotopes in paddy and wheat indicated that even the so-called tracer dosages have effects on cell division. In the legumes, *Vicia faba* was found to be more radiosensitive than others and amongst cereals, wheat was found to be more susceptible to beta-radiation than paddy. The factors contributing to such differential radiosensitivity are discussed.

Acknowledgement

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BOOK REVIEW

Proceedings of the International Symposium on Origin, Cytogenetics and Breeding of Tropical Fruits. Special Symposium Number of Indian Journal of Horticulture Vol. 15 (3 & 4)-1958. pp. vii+186. Price Rs. 20/-. (Copies can be had from Horticultural Society of India, Division of Horticulture, IARI, New Delhi-12, India).

An International Symposium on the Origin, Cytogenetics and Breeding of Tropical Fruits sponsored jointly by the Horticultural Society of India and the UNESCO South Asia Science Cooperation Office was held at the Indian Agricultural Research Institute, New Delhi from December 1 to 6, 1958. Distinguished horticulturists from United Kingdom, United States of America, Japan and Ceylon participated in the symposium besides the delegates from the different States of India.

The papers presented at this Symposium have now been published as a special number of Indian Journal of Horticulture. This number is divided into four sections, namely, origin of the tropical fruits, breeding of tropical fruits, cytogenetics and improvement of tropical fruits and physiological aspects. In the first section the distinguished Japanese taxonomist Prof. T. Tanaka traces the origin, and dispersal of citrus fruits having their centre of origin in India. The origin of mango is discussed by Dr. S. K. Mukherjee of India while Dr. C. A. Schroeder of California deals with origin and spread and improvement of avocado, sapodilla and papaya.

In the second section on the breeding of tropical fruits, the problem of crop improvement in mango is examined in detail by Dr. K. C. Naik and others. The Citrus breeding in progress at U.S.A. and Japan, has been reviewed by Dr. C. A. Schroeder and Prof. T. Tanaka respectively. The improvement of grapes and Annona also has been dealt with by Indian Horticulturists. Dr. K. S. Dodds of United Kingdom has discussed in detail the problems of banana breeding with particular reference to the pioneer work done at the Imperial College of Tropical Agriculture, Trinidad.

In the third section on cytogenetical aspect, an interesting and exhaustive discussion is given by Dr. K. S. Dodds, on the cytogenetical problems met with in different fruits; another article by the same author deals with incompatability systems in relation to fruit breeding. The techniques involved in the induction of mutations in fruit trees are examined in detail, with special reference to modern developments of irradiation with radioisotopes, by Dr. M. S. Swaminathan of India.

In the fourth and final section, some aspects of fruit tissue culture are explained by Dr. C. A. Schroeder of California and the problem of polyembryony in citrus and mango, has been dealt with by Prof. P. Maheshwari of India. A paper on the Citrus die-back problem in India has been contributed by Dr. R. D. Asana.

The papers presented at the Symposium cover all aspects of fruit breeding and some of the recent developments in fruit breeding that have taken place in different countries, have been discussed in relation to tropical fruits. A record of discussions has been appended with each paper. The issue carries also in the end the recommendations made by the Symposium for adoption by the Governments of the South East Asian region.

The get-up of the number is attractive and the issue is moderately priced, to suit the requirements of the different Universities, Agricultural Colleges and other Scientific Institutions.

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